Chapter 7
Other Chemical Hazards

Lijuan Du, Guoren Huang, Puyu Yang, Zhongfei Zhang, Lu Yu, Yaqiong Zhang, and Boyan Gao

This chapter reviews five groups of chemical hazards, including 5-HMF, trans-fatty acids, MCPDs and their esters, glycidol and its esters, and acrolein and other alkenals. Their analytical methods, formation mechanisms, and mitigation strategies are discussed. Understanding these chemical hazards may improve our knowledge about the whole thermal-processing-induced hazards, then improving food safety and quality in food industry.

7.1 5-HMF

5-HMF (IUPAC name, 5-(hydroxymethyl)furan-2-carbaldehyde; Fig. 7.1) was a ubiquitous food contaminant formed during processing of sugary foods. 5-HMF is mainly generated from hexose under high temperature or during long-term storage at room temperature, regardless of the presence of oxygen or UV light (Maillard reaction). Aberrant crypt foci were induced in a dose-dependent matter in F344 rats by 5-HMF [1] and skin tumor was reported in mice [2]. However, no genotoxicity could be detected from 5-HMF in standard in vitro assays, such as gene mutation assay [3], the rec assay [4], or comet assay [5]. To conform the acute, subacute, and

L. Du · G. Huang · P. Yang · Z. Zhang · Y. Zhang
Department of Food Science and Engineering, School of Agriculture & Biology, Shanghai Jiao Tong University, Shanghai, China

L. Yu
Department of Nutrition and Food Science, University of Maryland, College Park, MD, USA

B. Gao (✉)
Department of Food Science and Engineering, School of Agriculture & Biology, Shanghai Jiao Tong University, Shanghai, China

Department of Nutrition and Food Science, University of Maryland, College Park, MD, USA
e-mail: gaoboyan@sjtu.edu.cn

© Springer Nature Singapore Pte Ltd. 2019

S. Wang (ed.), *Chemical Hazards in Thermally-Processed Foods*,
https://doi.org/10.1007/978-981-13-8118-8_7

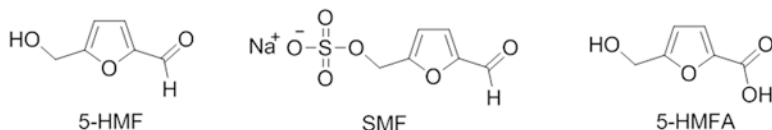

Fig. 7.1 The structure of 5-HMF, SMF, and 5-HMFA

chronic toxicity of 5-HMF, the National Toxicology Program conducted a toxicological evaluation on 5-HMF using F344/N rates and B6C3F1 mice for 3 weeks, 3 months, and 2 years. They concluded that 5-HMF caused liver cancer in female mice and was related with gradually enhanced injury of the respiratory epithelium and olfactory regardless of the gender and rats or mice [6].

In recent years, 5-sulphooxymethylfurfural (SMF, Fig. 7.1), the metabolite of 5-HMF, was confirmed with mutagenicity and genotoxicity by in vitro and in vivo studies [7, 8]. At the same time, 5-HMF was linked with the formation of 5-hydroxymethyl-2-furoic acid (5-HMFA) (Fig. 7.1) [9], acrylamide [10], dicarbonyl compounds [11], and melanoidins [12]. So, it is necessary to detect the amount, illuminate the formation mechanism, and discover the mitigation strategies of 5-HMF in food processing.

7.1.1 Analytical Methods

5-HMF is a needle-like crystalline solid with a slight odor of chamomile flowers. It tastes buttery, caramellic, and musty (data from HSDB) and is easily dissolved in water and methanol. 5-HMF has a maximum UV absorption wavelength at 283 nm. To remove the interference of protein which has strong absorption wavelength at 280 nm, Carrez clarification reagents were used [13]. The analytical methods for 5-HMF in foods will be discussed as two major approaches, the direct approaches and the indirect approaches [14–16].

7.1.1.1 Direct Approaches in Detecting 5-HMF

Direct approaches aim at analyzing 5-HMF directly without breaking the structure of 5-HMF. In 2009, melamine and 5-HMF were firstly detected by CE-DAD (capillary electrophoresis-diode array detection) in milk samples; the linearity range of 5-HMF was 0.1–100 μg/mL. The limit of detection (LOD) was 0.067 μg/mL for 5-HMF [17]; in 2010, Gloria del Campo et al. used ^{1}H NMR spectrometry to detect the 5-HMF and caffeine, formic acid, and trigonelline in soluble coffees; the LOD of 5-HMF was 0.30 mg/g; this method was no need for derivative procedure [18]; in 2013, Ales Rajchl used a novel technique series connection of direct analysis in real time (DART) ion source and time-of-flight mass spectrometry (TOFMS) to develop a rapid determination of 5-HMF. Linearity was measured in the range 1–1000 mg/L;

the calibration plot was linear only within the range 1–20 mg/L (R^2 = 0.9985). Repeatability of the measurement was 66% and 11% (RSD) without and with the internal standard (isotope-labeled 5-HMF), respectively. For the honey and caramel samples, the LOD was 2 and 3 mg/kg, respectively; the LOQ was 3 and 4 mg/kg, respectively. Recovery was 98% (concentration 10 mg/kg) and 101% (concentration 10 mg/kg) for honey and caramel samples, respectively [19]. This method requires isotope-labeled 5-HMF as internal standard and needs expensive TOF mass spectrometry. So, this method is difficult to have a wide application.

In 2016, Jucimara developed an HPLC-DAD method for quantitative analysis of HMF in cane syrups and corn. In this method, the column was C18 at 30 °C and DAD was set at 285 nm, and the mobile phase was acetonitrile/water (1/9, v/v, added with 0.5% formic acid) isocratic elution, and flow rate was 0.8 mL/min. The LOD was 0.09 mg/L and LOQ was 0.26 mg/L; the recovery rates were between 100% and 104%; RSD was 0.57–6.43%. The contents of HMF were 109.2–893.1 mg/kg and 406.6–2121.3 mg/kg in cane syrup and corn syrup, respectively [20]; in 2017, Terra and colleagues developed UV-MCR-ALS methods for quantification of 5-HMF with the use of ultraviolet (UV) spectroscopy technique in a combination with data analysis technology of multivariate curve resolution (MCR) coupled with alternating least squares (ALS). This model was evaluated by analyzing statistical parameters of quality such as root mean square error (0.68 mg/L) and correlation coefficient (R = 0.988). This method was easy and quick, and no pretreatment and chromatographic separations were required. In addition, it would achieve the accurate determination of 5-HMF content [21]. However, solid and fluid cannot be directly measured using this method. The food matrix is complex and different, so each kind of sample needs a separate modeling analysis. This method shows great potential in automation applications but still requires deepgoing research.

7.1.1.2 Indirect Approaches in Detecting 5-HMF

5-HMF has both hydroxyl and aldehyde groups, which could easily react with derivatization reagents to get specific target products. Colorimetric method was used to detect 5-HMF in tomato with the use of toxic derivatization reagents barbituric acid and *p*-toluidine. Now this method is gradually abandoned, because of the poor repeatability and anti-interference. In 2009, Bernhard et al. used UPLC-MS/MS to detect 5-HMF in plasma samples. Derivative reagent was 2,4-dinitrophenylhydrazine (DNPH) dissolved in acetonitrile/HCl (1 M, 3/1, v/v), equivalent volume mixed with samples and incubated for 1 h at 37 °C; used potassium hydroxide which dissolved in equivalent volume of water and ethanol to neutralize the redundant acid; finally diluted by water. After centrifugation, the upper layer was used for analysis. The derivative compounds of DNPH-HMF were analyzed by water MS in the positive ion mode with ESI [22]. The derivative process was complex and time-consuming and derivatization reagent is toxic for the analyst. So now the application of this method is relatively narrow.

7.1.2 Formation Mechanisms

5-HMF was found at high levels in many foods, especially from sugar-containing products processed under high temperature. HMF in foodstuff was mainly produced by two types of chemical reactions. The first reaction includes caramel products, instant coffee, biscuit, and other roasting foods. This group was considered as major sources to form 5-HMF mainly through caramelization reaction, whereas the second one includes dried fruits, fruit juices, and paste, which can form 5-HMF by Maillard reaction.

Antal and colleagues demonstrated a formation mechanism of 5-HMF from fructose using isotope labeling [24]. Fructose (A1) or sucrose was dehydrated at C5 position to form the fructofuranosyl oxocation, and then a derivative like enol of 2,5-anhydro-D-mannose was formed by losing a proton through enolization. In the next step, the structure was isomerized to aldehyde group and a second water molecule was released at C4 to form the double bond in the furan ring. When the third water molecule was eliminated at C3 position, 5-HMF was formed [25]. This pathway was shown in Fig. 7.2.

When the hexose was glucose, the mechanism of 5-HMF formation was more complex. Glucose may undergo complex transformation in which the intermediate was tautomeric enediol and isomerized into fructose through a tautomeric 1,2-enediol (A2), which was subjected to a dehydration step at C3 to form unsaturated hydroxyaldehyde (structure 6). 3-Deoxy-2,3-diulose (structure 7) might be automatically converted to unsaturated hydroxyaldehyde (structure 6), which was associated with numerous side reactions. Structure 7 released the second water molecule at C4 forming dicarbonyl compound (structure 8). Then, cyclization and dehydration resulted in 5-HMF formation [24]. This pathway was shown in Fig. 7.2.

The proteins and amino acids can take part in the formation of 5-HMF in food processing [26]. It is not clear whether fructose reacts with amino acid to form 5-HMF through a prolix pathway [27] or through isomerization into glucose. The formation mechanism of 5-HMF from glucose and amino acid was widely accepted (Fig. 7.3). This was similar to the beginning and middle stages of the Maillard reaction [23, 28].

7.1.3 Mitigation Strategies

For now, only honey and dairy products limited the amount of 5-HMF by legislation. For the foods such as coffee, biscuit, dried fruit, etc., there is no limit to the content of 5-HMF. At the same time, the toxicity mechanism of 5-HMF has not yet been elucidated. Although 5-HMF is common and abundant in the foods, it does not attract enough attention. So, the literature associated with mitigation strategies of 5-HMF is limited. The formation of 5-HMF was linked with Maillard reaction directly; as such the researchers pay more attention to mitigation strategies for reducing Maillard reaction and its toxicity derivate such as anti-Maillard agents [29]

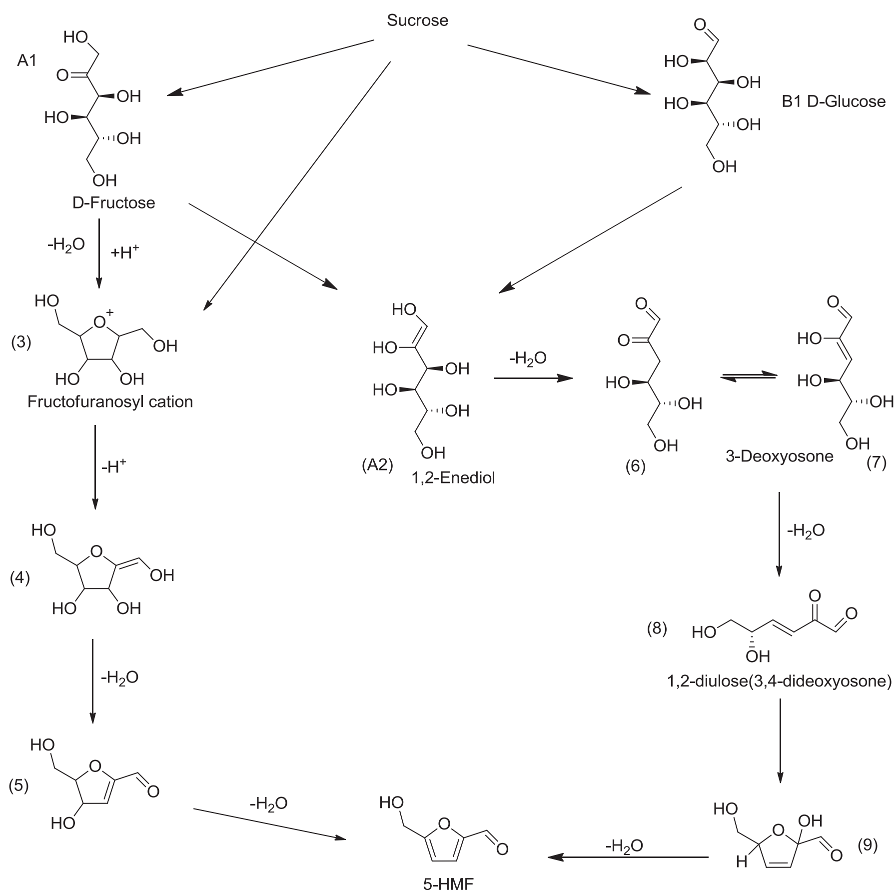

Fig. 7.2 Formation mechanism of 5-HMF from sucrose. (Adapted from Perez Locas (2008) and Stanistaw Kowalski (2013) [23])

or advanced glycation end product (AGE) inhibitor in recent years [30, 31]. These methods and strategies are worth being used for reference. Next, we will summarize the mitigation strategies to reduce 5-HMF in food matrix.

7.1.3.1 Optimization of Food Processing Conditions

The content of 5-HMF in food matrix was determined by temperature, time, pH, and activity of the water. During baking, when the temperature at the surface significantly increases, the 5-HMF could be rapidly accumulated [32]. The heating temperature and time are the decisive factors. When the pH value rises from 3.28 to 4.37 and then to 7.40, the level of formation of 5-HMF showed a significant decrease during baking at more than 200 °C [33]. Typically, relatively lower pH value will increase the formation of HMF in the biscuit during baking. In 2016, cocoa bean was immersed in 7.5% Na_2CO_3 containing the alkaline solution for 30 min, while

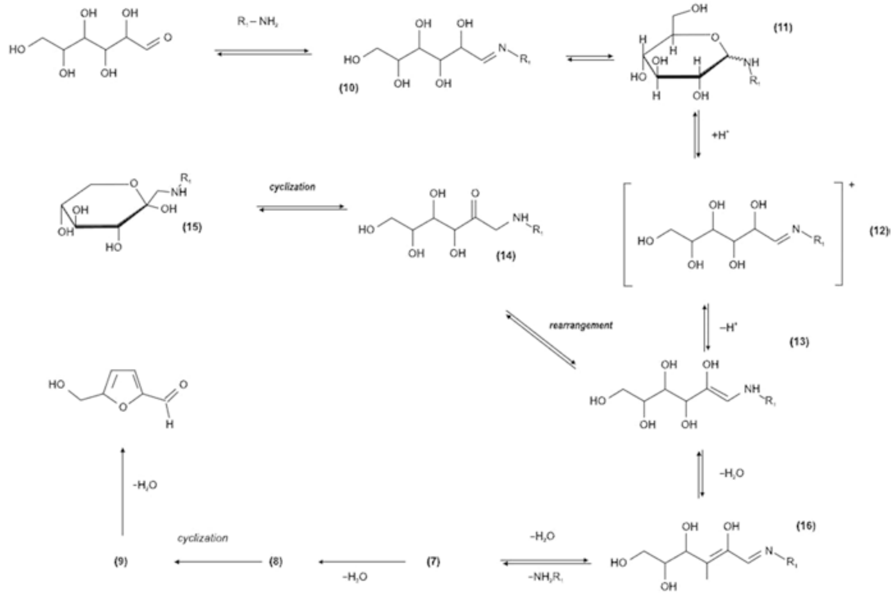

Fig. 7.3 The formation mechanism of 5-HMF from glucose and amino acid or protein. (Adapted from Kowalski (2013) [23])

water treatment and no treatment were used as a comparison. After being roasted for 1 h, 5-HMF concentrations were 0.34 mg/kg, 0.41 mg/kg, and 0.73 mg/kg in alkaline-treated samples, water immersed, and non-treated, respectively. The concentration of α-dicarbonyl compounds and N-carboxymethyl-L-lysine was higher for alkaline-immersed samples [34]. Both of them were more toxic than 5-HMF, so it needs comprehensive consideration when food processing conditions need optimization.

7.1.3.2 To Change Reactants from the Raw Materials

The sugar was also a key factor. Sucrose, glucose, fructose, and starch were common sugars used in food processing. At the baking temperature from 160 to 230 °C, the dough containing glucose produced more 5-HMF compared to sucrose [33]. When the temperature of baking biscuits exceeds 250 °C, using sucrose instead of glucose or fructose would result in more 5-HMF production [35]. In 1996, J. O'Brien compared the stability of trehalose, sucrose, and glucose in freeze-dried systems, with the presence of lysine at pH 2.5 and water activity of 0.33. After being heated at 90 °C for 194 h, trehalose system showed the lowest absorption at 420 nm and

280 nm [36], which means the trehalose was more stable than sucrose and glucose and less stable than furans (e.g., 5-HMF) generated. It is worth considering replacement of sucrose and glucose with trehalose in the processing of coffee, roasting foods, and dried fruit.

7.1.3.3 To Remove 5-HMF from Foods

Once 5-HMF is generated in the process of food processing, it is difficult to be removed. In 2017, T. Silva-Fernandes used two kinds of biopolymer, in which commercial names were Aquapol (tannin 18%, biopolymer A) and Bioclin (tannin 6.5–7.3%, biopolymer B) to remove 5-HMF from sugarcane bagasse hemicellulosic hydrolysates. The highest relative content removed by A and B biopolymer was 57% and 40%, respectively, after optimizing the operation parameters. From the above results, it can be seen that tannin-based biopolymers (TBBs) were efficiently and selectively adsorbed for removing 5-HMF. Because of the wide range of sources of tannins, TBBs were simple to prepare, low in cost, and environmentally friendly [37]. Therefore, other polymeric materials can be developed in the future to specifically adsorb 5-HMF.

7.2 Trans-Fatty Acid

Trans-fatty acids, as the name implies, have one or more double bonds in which the adjacent hydrogen is substituted by the opposite sides of the hydrocarbon chain [38]. Mono- and polyunsaturated fatty acid and conjugated linoleic acids are the important sources of dietary trans fats [39]. Fatty acid components seem to be a perennial concern of nutritionists and persons concerned with healthful diets. Previous researches have suggested a possible association of the intake of trans-fatty acids with the risk of coronary heart diseases. The concentrations of LDL cholesterol are increased and the contents of HDL cholesterol are reduced by the intake of trans-fatty acids. This has a negative impact on blood lipids [40].

Due to trans-fatty acid's higher melting point, the fluidity and permeability of cell membranes were altered and affected when trans fat incorporated into cell membranes [38]. The main target of this section is to provide a clear description of the relevant studies in trans-fatty acid, including their analytical detection method, formation mechanisms, and mitigation strategies.

7.2.1 The Analytical Detection Method of Trans-Fatty Acid

The negative impact on trans isomers of unsaturated fatty acids raised lots of concerns since the research consequence of controlled dietary intervention performed in 1990 [41–44]. These results indicated that *trans*-fatty acid (TFA) had a detrimental effect on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol. Based on a population-based studies in the USA, the consumption of trans isomers might affect the rate of coronary heart disease [45, 46]. Therefore, in order to understand the presence and content of TFA, it is particularly vital to develop different monitoring technologies. Owing to the complexity of the possible fat mixtures, TFA analyses were still challenges. Researchers used several techniques including gas chromatography (GC) [47], infrared spectroscopic (IR) [48], capillary zone electrophoresis (CZE) [49], and silver ion chromatography to determine TAF [50]. Among these detection technologies, GC is the most popular and common technique.

7.2.1.1 Gas Chromatography (GC)

Gas chromatography (GC) has been widely used in the fatty acid measurement of oilseed plant, human metabolism, and so on [47]. GC methods equipped with high-quality capillary columns for fatty acid analyses are allowed to get sensitive and reproducible results [47]. The fatty acid is transformed into suitable derivatives such as methyl esters, as required by analysis GC [51]. In principle, gas chromatography equipped with flame ionization detection (GC-FID) as the official method is applied to detect fatty acid because of its high resolution and sensitivity [47]. The authors proposed a quantitation method of TFA in vegetable and nonruminant fats and oils; the separation of *cis/trans*-octadecenoic (18:1) in hydrogenated vegetables was performed by the optimization parameters using the CP-Sil 88 and SP 2560 capillary gas chromatographic columns [52]. Because frying process is considered to be a source of TFA, Romero et al. compared TFA profile of potatoes fried in the extra virgin olive oil, high oleic sunflower oil, and sunflower oil from the frying 8 and 20 with frequent replenishment or without replenishment of used oil with fresh oil during the frying by GC [53]. Results represented that elaidic acid showed the greatest amount in fried potatoes. Huang et al. also measured the TFA content both in fried potatoes and in frying oils by GC [54]. The gas chromatography-mass spectrometer using Alltech AT™-Silar-90 capillary column could completely separate *trans*-fatty acid from *cis* standard. Moreover, under the optimized condition, shortening samples were analyzed to check the feasibility of this method; the predominant fatty acids detected in the sample were trans-18:1, cis-18:1, cis-18:2, and cis-18:3. In general, prior to GC analysis, the fractionation of fatty acids by liquid chromatography is applied to eliminate co-elution problems.

7.2.1.2 Capillary Zone Electrophoresis with UV Detection (CZE-UV)

Capillary zone electrophoresis (CE) is an identification and separation technique on the basis of solvated ions, neutral compounds, or ionizable species [55]. The technique as an attractive analysis method for fatty acid has been sought by the hundreds of other scientists. Compared with GC, CE is a throughput analytical method that employs the short analytical time and separated the analyses at lower temperatures without derivatization step [56]. According to the previous study, Oliveira and colleagues first reported a novel capillary electrophoresis with UV indirect detection for the TFA in hydrogenated oils [49]. It is suggested that the formation of TFA could be monitored by the optimized method. Later, CZE was applied for the different FA in different samples by the 2^3 central composite design optimization, owing to factors Brij 35, acetonitrile, and 1-octanol [57]. Moreover, the results were comparable with AOCS GC official method by t-test. However, these methods had the problems about partial dissolving of the capillary coating polymer in the background electrolyte (BGE), which could cause a significant interference in the separation of TFA. In this case, Porto and colleagues proposed a CZE methodology coupled with contactless conductivity detection using two different cyclodextrins in the BGE; it was used for the determination and quantification of TFA of processed food including in 13 min [58]. Furthermore, there were no marked differences of statistical results between CZE-UV and the classical GC method within the 95% confidence interval.

7.2.1.3 Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FT-IR), as a simplest analytical approach, is commonly applied for the measurement of TFA in the edible oils and fats. In general, the isolated TFA were quantified by FT-IR spectroscopy on the basis of the determination of *trans* peak in the area from 991 to 945 cm^{-1}, indicating CH out of deformation absorption [56]. Sherazi and colleagues used FT-IR techniques for the detection of TFA in cooking oil and hydrogenated oil samples [48]. The results obtained by the transmission FT-IR were comparable to GC-FID technique results and have indicated slightly better sensitivity and higher accuracy for low TFA values in the tested edible oil samples. Moreover, FT-IR with an attenuated total reflectance combined with partial least squares models was applied to analysis of TFA concentration of cereal products without oil extraction [59]. The predicted model of TFA has a higher coefficient of determination and lower standard error of performance, suggesting the superiority and robustness of predicting models for screening. The model-based FT-IR was closely related to the fingerprint region, which showed that the unique characteristic of TFA configuration at 966 cm^{-1} has the main contribution to the development of the PLS model [59]. The FT-IR of fats extracted from lipid samples extracted from cereal-based foods, analyzed with a

single-bounce horizontal attenuated total reflectance (SB-HATR), was developed to determine TFA content [60]. It can be verified that FT-IR combined with PLS model might be an effective method to predict TFA content in food.

7.2.1.4 Silver Ion Chromatography

Silver ion chromatography is recognized as one of the attractive techniques for *trans* monoenes profiling giving a good separation and reproducibility of TFA in dairy samples [61], which is most commonly used in the separation of saturated, *trans* monounsaturated, and *cis*-monounsaturated fatty acids along with *cis/trans* conjugated linoleic acid isomers [51]. Indeed, scholars generally believe that silver ion chromatography has no rival for discrimination of liquid species differing in the number and especially liquid with the configuration of double bonds [62]. Thin-layer chromatography, high-performance liquid chromatography, and supercritical fluid chromatography in the silver ion mode are rather powerful tools for the separation and determination of geometrical fatty acid composition [50]. Ag-TLC was performed to pre-fractionate *cis* and *trans*-fatty acids prior to accurately quantifying TFA by infrared spectroscopy and gas-liquid chromatography (GLC) [63]. The *cis* and *trans* geometric and positional fatty acid methyl ester (FAME) and triacylglycerol (TAG) isomer are separated and isolated by Ag-HPLC as alternative techniques [50]. Adlof investigated the profile of the *cis* and *trans* unsaturated FAME by Ag^+ high-performance liquid chromatography; the isocratic elution solvent was acetonitrile in hexane [64]. The other researchers also use Ag-HPLC with two commercially available columns connected in series as a separation method to analyze a mixture of conjugated 18:2 isomers [65]. The method provided an improved resolution of the *cis* and *trans* 18:2 isomer pair, but it could not resolve *trans* form of linoleic acid and linolenic acid.

7.2.2 The Formation Mechanism of Trans-Fatty Acid

The formation mechanisms of TFA are derived from biological hydrogenation in the stomach of ruminants and the industrial process of catalytic hydrogenation of fats. Of the dietary TFA, 80–90% are originated from the latter source, while 2–8% are offered by dairy products [66].

7.2.2.1 The Natural Sources from Ruminant Animals

It is well known that the digestion and absorption of dietary lipid for ruminant animals occurs in the reticulo-rumen [67]. Its metabolism process was comprised of hydrolysis of lipids, biohydrogenation of unsaturated fatty acids by rumen bacteria, and synthesis de novo of microbial lipids [67]. According to the previous studies

[68], the free fatty acids were liberated from hydrolysis of the dietary acryl lipids by microbial lipases in the rumen. Then unsaturated fatty acids suffered from biohydrogenation by rumen bacteria, and the end product of the hydrogenation becomes stearic acid (18:0), saturated fatty acids, *trans*-fatty acids, conjugated fatty acids, and its isomer [68]. The several studies basically confirmed that in hydrogenation pathways of rumen bacteria, the most common is *α*-linolenic and linoleic acid hydrogenation [67], as shown in Figs. 7.4 and 7.5. At first, both of them are formed to a conjugated *cis*-9, *trans*-11 acid by an initial isomerization step. Then, *cis*-9, *trans*-11 acid as intermediate product undergoes hydrogenation of its *cis* double bonds, while *trans*-11-octadecenoic acid is liberated as penultimate. In the end, they are hydrogenated to stearic acid. It is found that both *α*-linolenic and linoleic acid could be hydrogenated to *trans*-octadec-15-enoic acid by a rumen bacterium called *Butyrivibrio fibrisolvens*, which was directly involved in the metabolism of fatty acid [69]. In addition, the *Ruminococcus albus* could convert linoleic and linolenic acids to a mixture of octadecenoic acids and *trans*-octadec-11-enoic acids [68].

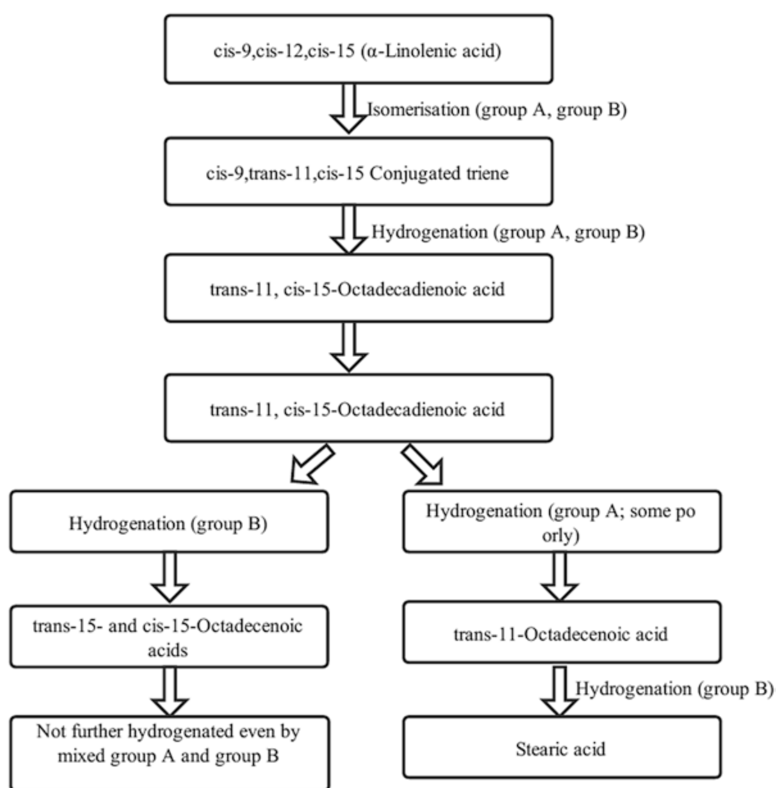

Fig. 7.4 Scheme for the biohydrogenation of linoleic acid; group A and B present the two types of biohydrogenating bacteria. (Adapted from Harfoot and Hazlewood [67])

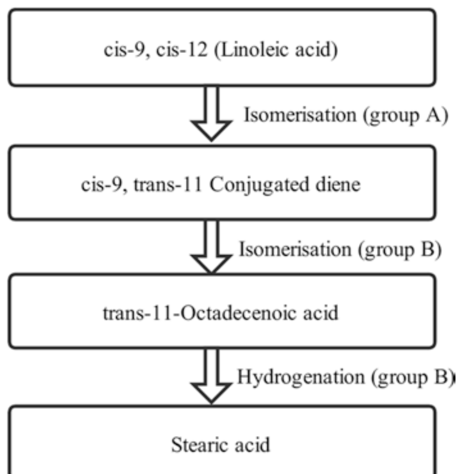

Fig. 7.5 Scheme for the biohydrogenation of linoleic acid; group A and B present the two types of biohydrogenating bacteria. (Adapted from Harfoot and Hazlewood [67])

7.2.2.2 The Processing of Hydrogenated Oil

Some fats and oils are susceptible to autoxidation or thermal degradation and other reactions during thermal food processing on the account of the presence of two isolated double bonds, in order to improve the stability and utility of fat or oils, which are often subjected to the process of hydrogenation, forming shortenings or margarine [70]. Therefore, the fat and oil hydrogenation is an indispensable operation in food and chemical industry. The process can convert a liquid oil to a solid or semisolid product by means of a multiphase catalytic reaction with hydrogen [71]. However, the occurrence of *trans*-fatty acid is a severe challenge in the process of oil hydrogenation.

According to the previous studies [72], the mechanism of geometric and positional isomers as described by Min, a free radical site, is formed when a hydrogen first entered either side of the double bond of unsaturated fatty acid, especially bound to the catalyst, while the free radical site is relatively unstable. A hydrogen atom neighboring carbon could be eliminated with the catalyst partially covered by hydrogen, thus regenerating the double bond or resulting from the formation of a positional isomer. The formed double bond may present as either *cis* or *trans* configuration because of free rotation properties of a free radical site (Fig. 7.6).

7.2.3 The Mitigation Strategies of Trans-Fatty Acid

The contents of TFA in some animal products occurred naturally in the fermentation step, and the amounts were relatively low. Thus, diet content of a large proportion of TFA is generally derived from partial hydrogenation of fats. In addition, the main dietary sources of TFA are fried and baked foods, traditional vegetable shortenings, and solid margarine. Excessive consumption of TFA can increase risk of heart

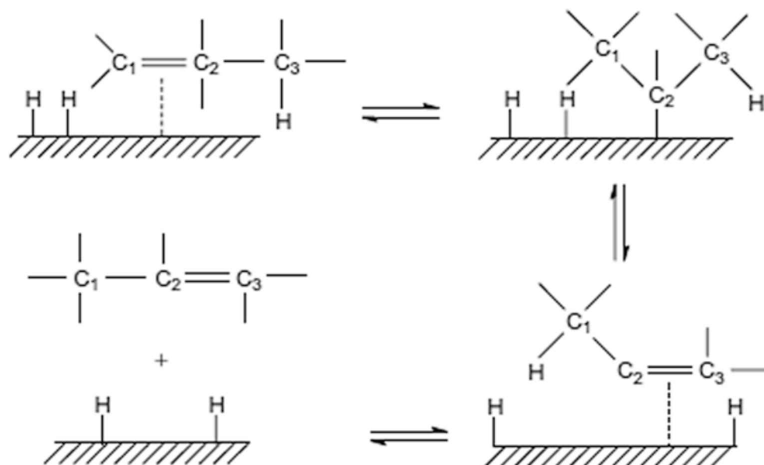

Fig. 7.6 Formation mechanism of geometric and positional isomers in the hydrogenation process

disease [73]. Therefore, food producers try their best to decrease the level of TFA in related products. Meanwhile, the authorities have made law and take some measure to prevent or decrease the damage of TFA.

7.2.3.1 The Method of Reduced TFA

Recently, several food manufacturers voluntarily removed TFA from their products. For example, in 1994, Unilever set about removing TFA from all retail margarine by introducing a worldwide policy [74], triggering by media coverage that TFA was more unhealthy than saturated fats [45]. In addition, many manufacturers have sought to TFA substitutes. In order to minimize the TFA content of the products, numerous technologies have been performed by the food and edible oil industries. Firstly, the partial hydrogenations were replaced by full hydrogenation to obtain fats with low TFA levels. Secondly, the oil seeds with modified fatty acid components are produced by traditional plant breeding and modern genetic engineering techniques for the sake of altering the oil properties [75]. Thirdly, the fractionated tropical oils are used in the manufacturing process [75]. Finally, the chemical and enzymatic interesterification of hydrogenated fats with liquid oils is obtained to a range of melting point customized fats to fit different food industry applications [75].

7.2.3.2 Regulating the Concentrations of TFA in Foods

Regarding the need for public health action of international policy consensus, TFA intake should be no more than 1% of total energy intake according to Physical Activity and Health, 2004 WHO Global Strategy on Diet [76]. Moreover, to reduce

the health risk associated to TFA, no more than 2 g/100 g fat on industrially produced TFA is imposed in Denmark [77], as labeling was inadequate to protect the consumers, particularly for children or people with high intake of fast foods. The Danish Nutrition Council requests a reduction of TFA in the cooking oil for major fast food. Based on Denmark's legislation introduced and enforced on June 1, 2003, the industrially produced TFA content of all food products and ready meals is restricted to a maximum of 2% [78]. Afterward, hydrogenated oils or margarine containing trans fats are illegally used to prepare food, except for foods that contain no more than 0.5 grams of artificial *trans* fat per serving of food [79]. In 2008, Switzerland begins to regulate TFA content. A survey determined by the Federal Institute of Technology has found that almost one-third of 120 food items contained excessive amounts of TFA. This finding was a key factor in attracting attention of the authorities and food industry to the problem [80].

7.3 MCPDs and Their Fatty Esters

Monochloropropane-1,2-diols (MCPDs), including 3-monochloropropane and 2-monochloropropane, are one type of chemical compounds mainly produced during thermal processing of food. MCPDs were recognized as potential food source toxicants in the past decades. Monochloropropane-1,2-diol fatty acid esters (MCPD esters) are a group of toxicants formed during food thermal processing. These compounds have been detected in many food categories, including refined edible oil, fried food, infant foods, and even in human breast milk. In 2004, 3-MCPD esters, as well as the free 3-MCPD, were firstly reported in processed foods. The ester form of 3-MCPD occurs at a much higher concentration in foods, especially in some high-lipid content foods after high-temperature processing. Therefore, a tolerable daily value calculated as the amount of free 3-MCPD at 2 μg/kg body weight was estimated by the European Food Safety Authority in 2013. These facts suggested that MCPDs together with their fatty acid esters are an important food safety concern and warrant research of their analytical methods, formation mechanisms, and the approaches for mitigation (Fig. 7.7).

7.3.1 Analytical Methods

Since MCPDs and their fatty acid esters were reported and considered as potential food source toxins in 2006 [81], it is important to develop accurate, fast, and high-sensitivity chemical analytical methods to detect this group of chemical components in different types of food samples. All of the reported analytical methods can be separated into two major types. The first method is to hydrolyze all the fatty ester forms of MCPDs to free MCPD first, quantify the amount of free MCPD, and then use the concentration of free MCPD to represent the total amount of all the MCPD

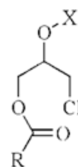

Fig. 7.7 Chemical structure of 3-MCPD and 3-MCPD esters

esters indirectly. This approach is much convenient to process. Another possible method is to characterize and quantify every MCPD ester in food samples directly. This method is straightforward and easy to understand, but is also much difficult for sample purification and method development. In the following paragraph, a systematic review of these two methods will be given to improve the understanding of the chemical analytical methods about MCPD esters.

7.3.1.1 Indirect Approaches in Detecting MCPD Esters

The basic working mechanism of the indirect analytical method starts with the transesterification of all of the MCPD esters into free MCPD under acidic or alkaline conditions, then free MCPD is transformed into a stable voltaic derivate, and, finally, the amount of free MCPDs is detected using GC or GC-MS [82]. This approach could also be used to analyze the free MCPDs. The major differences between these methods are the choices of different transesterification conditions and derivatization agents. In 2006, Zelinková and colleagues reported their study results about the quantification of 3-MCPD esters and free 3-MCPD in 25 vegetable oil samples using the indirect analytical approach [81]. In this study, 3-MCPD ester extracts were hydrolyzed using sulfuric acid, neutralized with a saturated $NaHCO_3$ solution, derivatized by using the phenylboronic acid, and then analyzed with GC-MS. The results indicated that most of 3-MCPD that existed in oil samples is bounded 3-MCPD (3-MCPD esters) rather than free 3-MCPD and 3-MCPD diesters are the major form of 3-MCPD esters. The results in this study also made the research about ester form of 3-MCPD become more and more important, since they are the major existence form of 3-MCPD in almost all types of foods. In 2008, Zelinková reported the occurrence of 3-MCPD esters, firstly, in human breast milk using a similar analytical method, which confirmed the stability of this method [83]. In the same year, Seefelder designed and processed a novel enzymatic hydrolysis method to hydrolyze the 3-MCPD esters to the dissociated 3-MCPD by using the intestinal lipase, followed by the GC-MS analysis [84]. The results represented the fact that only equal or less than 15% of 3-MCPD bounded in esters are monoesters and the rest of the parts are diesters. In 2008, Weißhaar reported a method to quantify 3-MCPD esters in edible fat and oil samples by transesterifying 3-MCPD esters with NaOCH3/methanol and derivatizing with phenylboronic acid, and finally, 3-MCPD was determined by GC-MS [85]. In 2010, Baer and colleagues published

a review article about 3-MCPD in food [82]. In this review, different derivatives including heptafluorobutyryl imidazole (HFBI) [86], phenylboronic acids (PBA) [87–89], and dioxolane were utilized to increase the volatility of hydrolyzed 3-MCPD. In 2016, Samaras and colleagues reported an indirect analytical method for the quantification of 3-MCPD esters, 2-MCPD esters, and glycidol esters in different types of food samples which were purified with pressurized liquid extraction (PLE) and determined using GC-MS. In order to differentiate glycidol esters from MCPD esters, all the glycidol esters were converted to monobromopropanediol esters (MBPD esters) in acidic solution first. Then MCPD esters and MBPD esters were hydrolyzed to release their free forms in ethyl acetate in the environment of phenylboronic acid. And the concentrations were finally quantified using isotopic labeled MCPD esters by GC-MS [90]. Also in 2016, an indirect analytical method using the enzymatic mechanism was applied to analyze 3-MCPD esters, 2-MCPD esters, and glycidol esters in vegetable oils and fats. This enzymatic method utilized *Candida rugosa* lipase to hydrolyze the ester form of MCPDs and glycidols to their free form at an ambient temperature for 30 min. Then the free form of MCPDs and glycidols was analyzed using GC-MS [91]. All these results above indicated that the indirect approaches required less analytical standards, thus recommended to be used to describe the total amount of 3-MCPD-related components in food matrix. But, on the other hand, the indirect methods need more sample preparation approaches with the use of some toxic chemical reagents. Also, the indirect method could only be used to quantify the total amount of 3-MCPD esters instead of every specific 3-MCPD ester. These defects made the indirect analytical methods more and more uncommon after 2010, and increasing researches turned to develop direct analytical methods to determine the concentrations of each 3-MCPD ester individually.

7.3.1.2 Direct Approaches in Detecting 3-MCPD Esters

Direct approaches aimed to develop chemical analytical methods to analyze 3-MCPD esters directly without hydrolyzing them to free 3-MCPD. This method was not widely applied before 2010. It might be majorly due to the nonpolar and relatively low volatile properties of 3-MCPD esters. As such they are not suitable for either LC or GC analysis. However, with the development of analytical techniques and new types of LC columns, there is increasing research about the direct approaches of analyzing 3-MCPD esters directly in different food matrixes. In 2012, Dubois and colleagues made a comparison of previous indirect methods with their novel developed a direct method to analyze 3-MCPD esters in edible vegetable oils [92]. In this study, double solid-phase extraction (SPE) and silicone gel column were utilized to separate and concentrate 3-MCPD esters; an HSS T3 normal-phase LC column was utilized with the combination of ESI time-of-flight (TOF) mass spectrometry. The comparison between indirect and direct methods represented that these two types of analytical methods showed similar results when analyzing 29 oil samples. The indirect approach needs fewer chemical standards and is relatively

easier for sample preparation and is applicable to all types of commodities compared to the direct approach which determines the total 3-MCPD ester content. On the other hand, direct method can provide detailed information about the identification and concentration of every 3-MCPD ester, though with a longer sample pretreatment time. After 2012, increasing research articles reported modified direct method of analyzing the types and concentrations of 3-MCPD esters in food samples or organ/tissue samples in in vivo studies [93–96]. All these studies selected high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) combined with high-resolution mass spectrometry to detect 3-MCPD esters directly; these methods represented the development of 3-MCPD ester analysis research and played an important role in the analysis of MCPD and its esters.

7.3.2 Formation Mechanisms

The formation mechanisms about MCPDs were first described as protein hydrolysates in 1978 [97]. In these years, increasing studies indicated that thermal processing might be closely related to the amount of MCPD esters in food products. And the ester form of MCPDs can be degraded to the free form of MCPDs. During this progress, the temperature was the most important factor that controls the formation of MCPDs. Take the refined edible oil as an example; deodorization was recognized as the key step in forming MCPD esters during processing, with the working temperature at around 260 °C. Reducing the temperature significantly decreased the concentrations of MCPDs. In order to clarify the formation mechanisms of MCPDs during food processing, different formation pathways have been derivated to explain the possible ways of ester form of MCPDs formed during thermal processing.

The primary aim about the studies of 3-MCPD esters is to investigate the chemical and biochemical formation mechanisms during food processing, since it is the fundamental of further research of 3-MCPD esters. Several previous researches investigating the possible formation mechanisms of 3-MCPD mono- and di-fatty acid esters were reviewed and summarized in four major possible types [98], including the direct nucleophilic substitution of chlorine anion of a hydroxyl (pathway 1) or a fatty acid ester group at sn-3 carbon atom in the glycerol (pathway 2) and through the formation of an acyloxonium cation (pathway 3) or through an epoxide cation intermediate (pathway 4) and then the intermediate cation attacked by chlorine anion to open the three- and five-membered rings to form MCPD esters (Fig. 7.8).

In 2013, a novel research approach about 3-MCPD ester formation from diacylglycerol was reported [99]. For the first time, this study reported a free radical intermediate reaction mechanism of 3-MCPD diester. In 2015, possible formation mechanisms of 3-MCPD monoesters and diesters from triglycerides were derivated and involve either a glycidol ester radical or a cyclic acyloxonium radical intermediate in a high-temperature and low-moisture condition [100]. In 2016, a further study

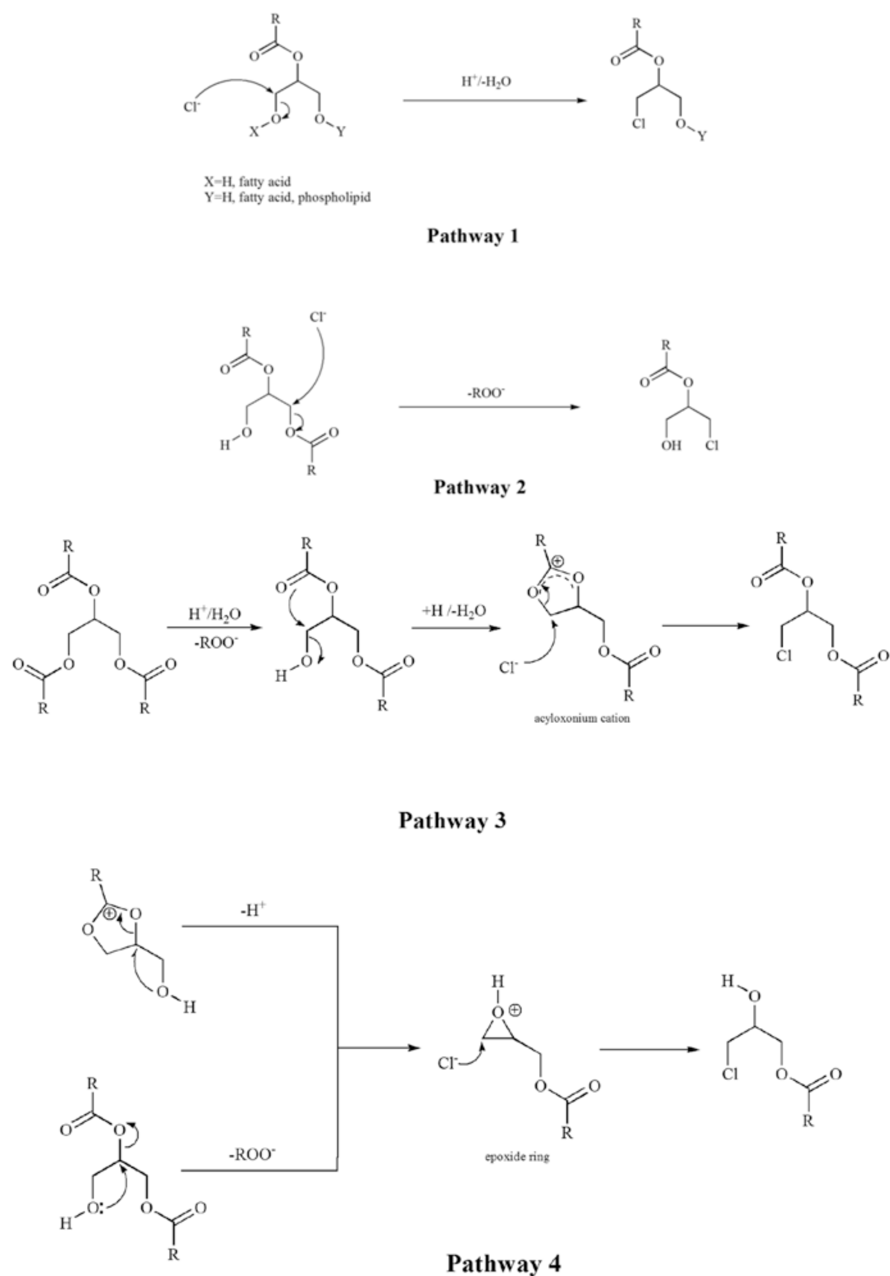

Fig. 7.8 Hypothesis of formation mechanisms of 3-MCPD esters

about the formation of 3-MCPD esters from monoglycerides was reported [101]. The results of this study confirmed the free radical intermediate reaction as one of the mechanisms for 3-MCPD ester formation. This study demonstrates for the first time that five- or six-membered cyclic acyloxonium structures might work as an intermediate in this free radical reaction, which could be used to explain the high-temperature and low-moisture formation environment of 3-MCPD and 2-MCPD esters during thermal processing. Besides, results also represented that the monoester of 3-MCPD could degrade to form monoacylglycerol or free 3-MCPD and the ferric ion might play an important role in the thermal degradation of 3-MCPD monoesters.

7.3.3 Mitigation Strategies

After clarifying the formation mechanisms of MCPD and 3-MCPD esters during thermal processing, the strategies for reducing the content of these compounds in foods become necessary and important. To our best knowledge, eliminating chlorine-related compounds in the deodorization is one of the most important steps in developing a mitigation strategy to reduce the existence of 3-MCPD ester during food processing. All these strategies could be summarized into two types: to remove the reactants (either the glyceride or the chlorine substances) before the formation of MCPD/MCPD esters or remove the MCPD/MCPD esters after their formation.

Another possible approach to mitigate the formation of MCPD esters is to remove or reduce major reactants from the raw food materials before thermal processing. For example, monitoring and controlling the existence of chlorine-related components in food materials could effectively mitigate the content of the MCPD esters.

Chelating agents are another group of compounds that can theoretically mitigate MCPD esters during thermal processing. Based on the free radical formation mechanism of MCPD esters, the existence of free radical intermediate is the key step for the formation of MCPD esters. Chelating agents could competitively react with the free radical intermediate products and reduce the amount of final products. Zhang and colleagues reported the results about using EDTA-2Na to competitively inhibit the generation of 3-MCPD esters during thermal processing [100]. These results represented a fact that chelating agents might be used in selected processing steps to effectively reduce the content of 3-MCPD esters in the thermal processed food.

Although MCPD esters were formed during thermal processing, latest studies reported a potential catalytic role of ferric ion in degradation of 3-MCPD monoesters during thermal treatment [101]. In this study, 3-MCPD monoesters could be degraded at a greater ratio with the existence of ferric ion; meanwhile glycidyl esters and monoglycerides were formed significantly.

Besides, there are some other strategies that might be used to reduce the concentrations of MCPD/MCPD esters in food during thermal processing, such as manipulating the deodorization conditions or using lower chlorine-contaminated fats instead of higher chlorine-contaminated fats [102]. All these approaches aim to mitigate the existences of MCPD/MCPD esters in thermal processed foods.

7.4 Glycidol and Its Fatty Acid Esters

Glycidol has been classed as a probable carcinogen in humans (2A) (IARC, 2000), due to the alkylation properties of the epoxide prone to react with cellular nucleophiles which induced the mutagenic and carcinogenic effects in rodents. Glycidol fatty acid esters (GEs) (Fig. 7.9) have been detected in a variety of thermal processing food, especially in refined oil [103–105]. In the gastrointestinal tract, GEs can hydrolyze to form the free glycidol [106, 107]. Therefore, GEs are also probable carcinogen in humans, which need significant effort to inhibit and eliminate. The section below aims at the following topics: (i) analytical method for glycidyl and its ester detection, (ii) chemical mechanism for the formation of glycidyl and its esters, and (iii) possible approaches to reduce glycidyl and its ester levels in foods.

7.4.1 Analytical Method for Glycidyl and Its Ester Detection

Glycidol can be detected in gas chromatography combined with flame ionization detection [108]. And the analytical method for GE detection could be summarized into two major approaches, indirect methods and direct methods.

7.4.1.1 Indirect Analysis Methods

In the indirect analysis methods of GEs, the GEs are hydrolyzed into glycidol and then glycidol derivatized with derivatizing agents such as phenylboronic acid (PBA), heptafluorobutyryl imidazole (HFBI), bis(trimethylsilyl)trifluoroacetamide (BSTFA), or heptafluorobutyric anhydride (HFBA).

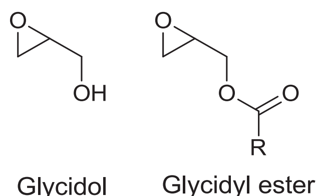

Fig. 7.9 Structure of glycidyl and glycidyl esters

The German Society for Fat Science (DGF) official method (DGF Standard Methods C III 18 (09) 2009) is an indirect analytical method. This indirect analysis method consists of two pretreat options (options A and B) in different analytical mechanisms. In brief, GE samples are hydrolyzed in either acidic or alkaline conditions to free the glycidol and purified by a liquid-liquid extraction, derivatization with derivative reagents, and followed by the GC-MS quantification. Finally, the total GE amounts are calculated as the measured amounts multiplied by 0.67 (stoichiometric factor). Based on the DGF official method, a lipase from *Candida rugosa*-catalyzed hydrolysis of GEs for 30 min at room temperature shortens detection time [91, 109].

Three indirect analysis methods of GEs in fats and oils have been published by American Oil Chemists' Society (AOCS), namely, AOCS Cd29a-13, Cd29b-13, and Cd29c-13 [110–112]. In mildly alkaline or lipase-catalyzed conditions, GE samples transesterification, then transformation into monobromopropanediol (MBPD), derivatization of MBPD with derivative reagents, and followed GC-MS analysis.

Kuhlmann reported a new type of indirect analysis methods of GEs and 3-MCPD esters, which could analyze the glycidol derivatives and 3-MCPD derivatives independently [113].

Although the lipase-catalyzed conditions in indirect determine methods enables GEs, 3- and 2-MCPD esters to be analysis simultaneously with and the lipase use for the hydrolysis of esters, averting the transformation of MCPD esters or partial acylglycerols into GEs [109, 114], but in the bromination process, an incomplete bromination condition may still produce an underestimation amount of GEs in the tested oils samples. All these reasons above made the direct determination method without transesterification or derivatization necessary.

7.4.1.2 Direct Analysis

The direct methods include liquid chromatography-mass spectrometry (LC-MS), GC-MS, and nuclear magnetic resonance (NMR). Haines and his colleagues described the determination of seven kinds of GEs in edible oils by using LC-TOFMS in the positive ion mode [106]. And the sample preparation was the simplest to implement: the edible oil samples were diluted in a mixed solvent (methanol-sodium acetate solution (0.26 mM)/methylene chloride/acetonitrile = 1/8/1) prior to analysis. The LOD of glycidyl myristate in refined edible oil was estimated to be 0.29 mg/kg, and the LODs of other six GEs were 0.1 mg/kg. Based on the LC-MS method, Shiro and his colleagues reported a more sensitive LOD, which is only 1/3 compared to that reported by Haines and his colleagues [115]. Leigh and his colleagues developed an LC-MS/MS method to detect seven kinds of GEs [116] by liquid-liquid extraction. In increasing the formation of sodiated adducts, Hori and his colleagues added the sodium salts in the mobile phase, which might induce a significant negative impact on MS instruments [117]. Prior to LC-MS analysis, several purification techniques are used to remove the large amount of tri- and partial acyl

glycerides, including gel permeation chromatography (GPC) [118] and two-step SPE [119]. Steenbergen and his colleagues have reported a novel direct analysis approach for intact GEs in edible fats and oils: the sample was extracted by acetonitrile and heptane and then purification by normal-phase liquid chromatography, followed by GC-MS analysis [120]. The direct analysis method for GEs based on ^1H NMR spectroscopy was reported by Song and his colleagues [121]. In this study, the quantification formula of GEs was deduced from the characteristic diagnostic signals of two epoxy methylene (CH_2) protons, in which chemical shifts were 2.56 and 2.76 ppm, respectively.

To sum up, the advantage of the indirect analysis method of GEs is the simple and unique standard. On the other hand, the direct methods could supply more detail information about the chemical profiles of GEs in samples directly. Therefore, the direct analysis method could be an alternative method for the routine analysis of GEs.

7.4.2 Chemical Mechanism for the Formation of GEs

Till now, no systemic study was reported about the formation mechanism of glycidol in the thermal processing of food. But there are a few studies which reported the theoretical approaches about the chemical mechanisms for the formation of GEs using standard chemical compounds as a model.

DAGs and MAGs are common substrates for the formation of GEs; TAGs and MCPD esters also transferred to GEs under some condition. This chapter will review the formation mechanisms of GEs from MAGs, DAGs, TAGs, and MCPD esters.

There are three proposed chemical mechanisms of the formation of GE from MAGs (Fig. 7.10). One of them considers a direct elimination of water for 1-MAGs [101]. Another one of them considers an elimination of water after a direct intramolecular rearrangement for 1-MAGs [122]. The last mechanism involves the formation of the cyclic acyloxonium ion (CAI) by dehydration of either 1-MAGs or 2-MAGs (MAGs) and then elimination of water for CAI after the intramolecular rearrangement [98, 123, 124].

There are also three proposed chemical mechanisms for the formation of GE from DAGs (Fig. 7.11). All three proposed mechanisms involve an intramolecular rearrangement via charge migration, and differences among the three mechanisms are the nature of the intermediate and the leaving group. One of them considers an elimination of fatty acid for either 1,2-DAGs or 1,3-DAGs (DAGs) after a direct intramolecular rearrangement (Fig. 7.11, pathways a and a′) [125]. The other mechanism involves the formation of the CAI by deacidification of 1,2-DAGs (Fig. 7.11, pathway b) [98, 123, 124]. The last proposed mechanism involves the formation of

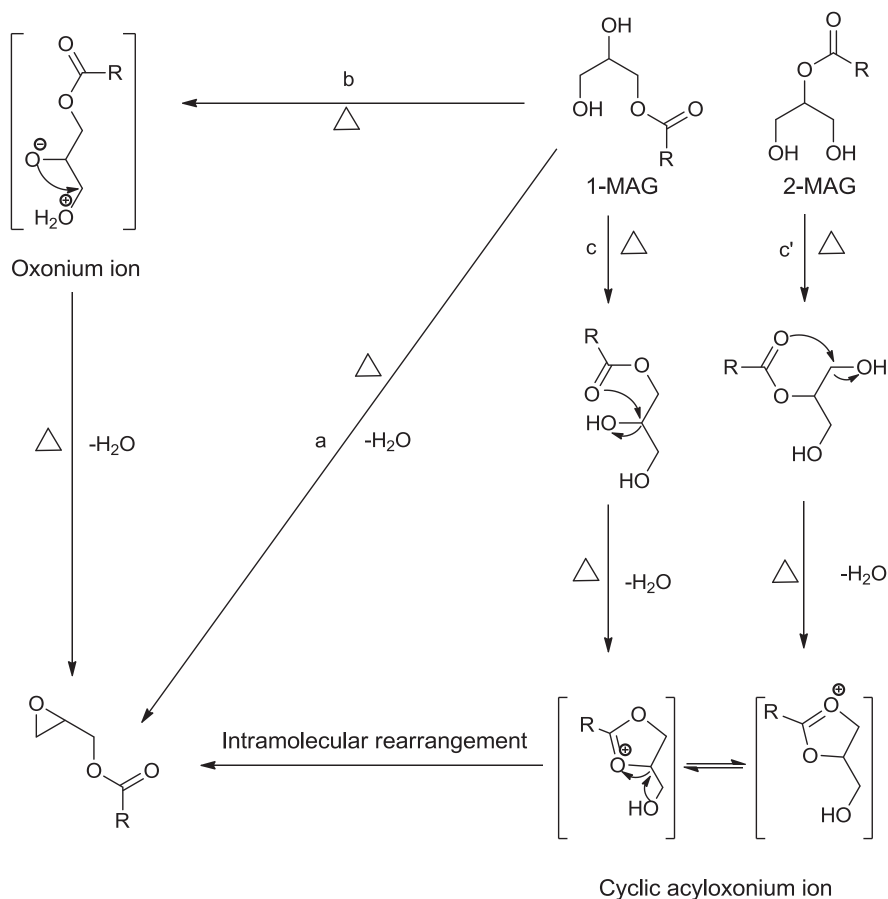

Fig. 7.10 Summary of proposed chemical mechanisms of GE formation from MAGs. Pathway (a), direct elimination of water for 1-MAGs [101]. Pathway (b), direct intramolecular rearrangement form of oxonium ion followed by elimination of water from 1-MAGs. Pathways (c) and (c′) involve the formation of the cyclic acyloxonium ion by dehydration of either 1-MAGs or 2-MAGs (MAGs) and then elimination of water for CAI after the intramolecular rearrangement [98, 123, 124]

an intermediate which is known as cyclic acyloxonium free radical (CAFR) by either deacidification (Fig. 7.11, pathway c) or dehydration (Fig. 7.11, pathway c′) of 1,2-DAGs [124].

There are two proposed chemical mechanisms for the formation of GE from TAGs (Fig. 7.12). One of the mechanisms involves the formation of either DAGs or MAGs derived from TAGs and then transforms GE by known pathway of DAGs or MAGs (Fig. 7.12, pathways a and a’) [98, 124]. The other proposed mechanism involves the formation of the CAFR intermediate by deacidification of sn-2 and sn-3 fatty acid (Fig. 7.12, pathway b) [100].

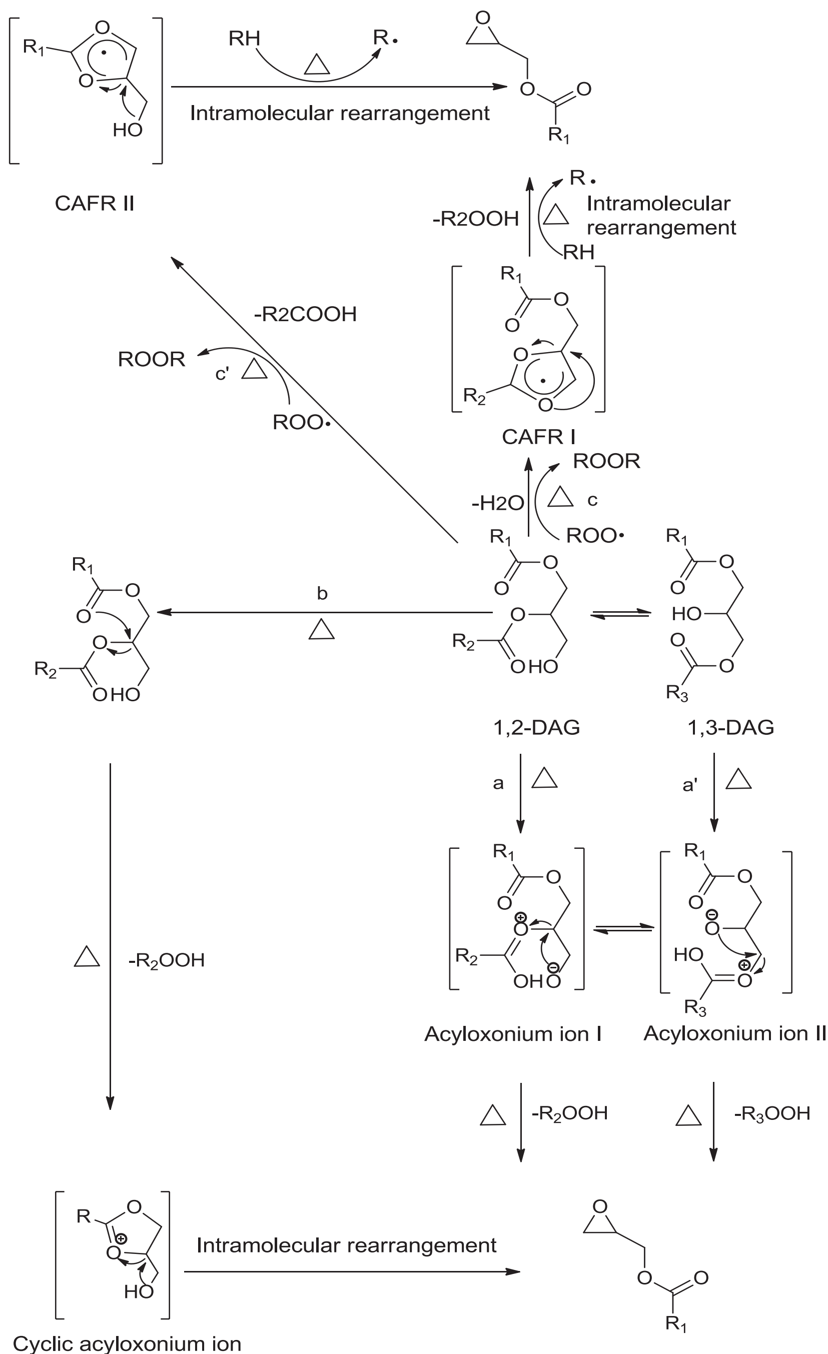

Fig. 7.11 Summary of three proposed mechanisms of GE formation from DAGs. Pathways (a) and (a′), an elimination of fatty acid for either 1,2-DAGs or 1,3-DAGs after a direct intramolecular rearrangement [125]. Pathway (b) involves the formation of the intermediate which is known as cyclic acyloxonium ion (CAI) by deacidification of 1,2-DAGs [98, 123, 124]. Pathways (c) and (c′) involve the formation of an intermediate which is known as cyclic acyloxonium free radical (CAFR) by either deacidification (pathway c) or dehydration (pathway c′) of 1,2-DAGs [124]

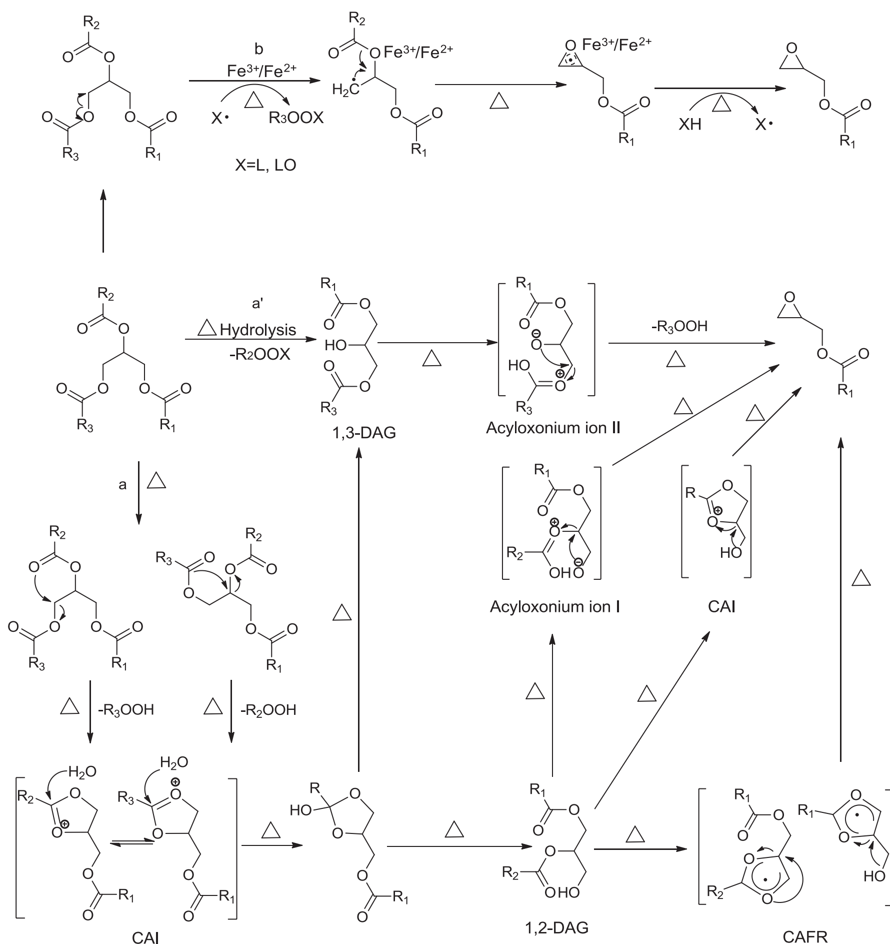

Fig. 7.12 Summary of proposed mechanisms of GE formation from TAGs. Pathways (a) and (a′) involve the formation of either DAGs or MAGs derived from TAGs and then transforms GE by known pathway of DAGs or MAGs [98, 124]. Pathway (b) involves the formation of the CAFR intermediate by deacidification of sn2 and sn-3 fatty acid [100]

There are two proposed chemical mechanisms for the formation of GE from 3-MCPD esters (Fig. 7.13). One of the mechanisms involves the formation of the alcoholate intermediate derived from either monoesters of 3-MCPD (a) or monoesters of 2-MCPD (a′) in alkaline media (Fig. 7.13, pathways a and a′) [106, 124]. The other proposed mechanism involves the formation of the carbocation intermediate derived from 3-MCPD monoesters in neutral and acidic media (b) or 2-MCPD monoesters (b′) (Fig. 7.13, pathways b and b′) [106, 124].

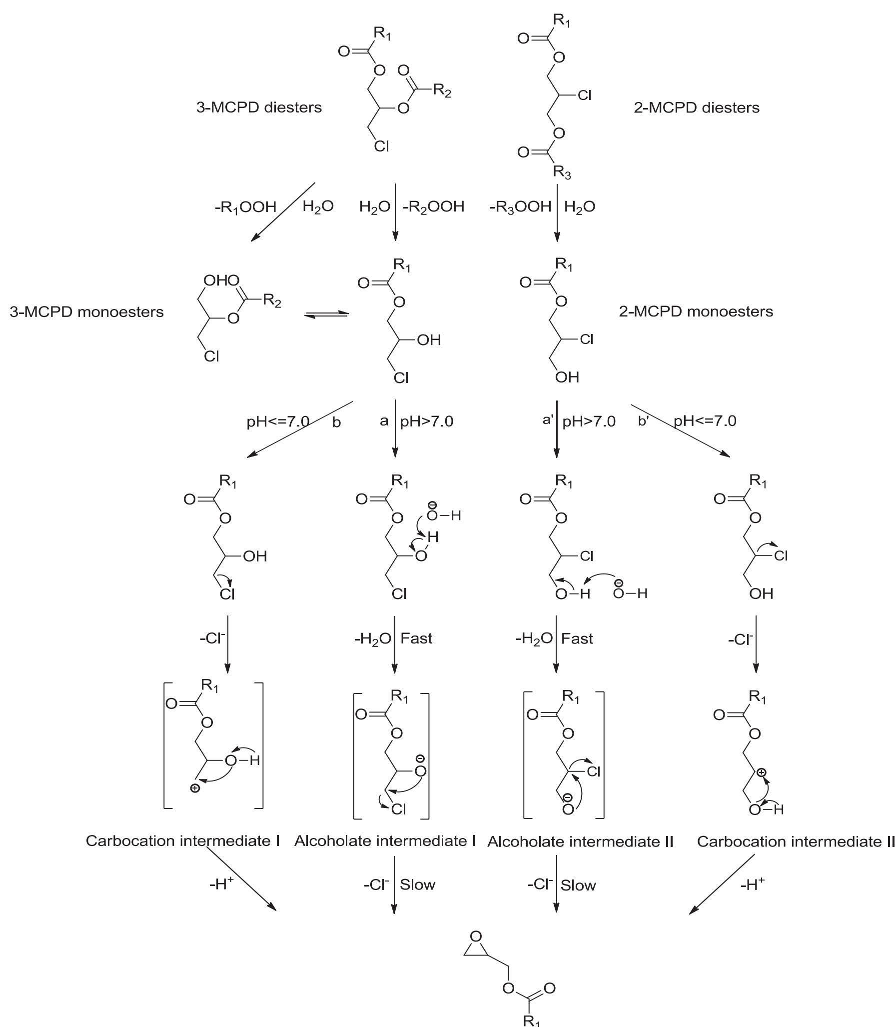

Fig. 7.13 Summary of proposed mechanisms of GE formation from MCPDs. Pathways (a) and (a′) involve the formation of the alcoholate intermediate derived from either monoesters of 3-MCPD (a) or monoesters of 2-MCPD (a′) in alkaline media [106, 124]. Pathways (b) and (b′) involve the formation of the carbocation intermediate derived from 3-MCPD monoesters in neutral and acidic media (b) or 2-MCPD monoesters (b′) [106, 124]

7.4.3 *Possible Approaches to Reduce Glycidyl Ester Levels in Foods*

7.4.3.1 Inhibition and Removal of Reagents

As shown in formation mechanism parts, the reagents of GEs are mainly DAGs and MAGs, and the TAG and MCPD esters should not be ignored. There are only a few approaches about reducing DAGs and MAGs to inhibit GE formation.

The removal of the main reagents (DAGs and MAGs) is the most efficient method to control the formation of GEs. For edible vegetable oil producers, the climatological locations, soil fertility and planting conditions, the harvest and processing practices of oil fruits/seeds, damaged oil seeds/fruits, and postmature fruits are essential to activate lipase and then change the content of DAGs and MAGs in oil seeds/fruits [104, 126–128]. All these factors could reduce the DAG and MAG content in oil. For the reason of inhibiting the lipase activity, the sterilization temperature during the milling of oil fruits/seeds should be kept at or below 120 °C [129, 130]. Aniołowska and Kita also found that the composition of oil fruits/seeds had a greater influence on polymerization transformation and GE formation than on hydrolytic and oxidative reaction in the frying oil [131]. Another strategy is removing some of the DAGs and MAGs in the refining steps (degumming, neutralization, bleaching, and deodorization) [105]. Some adsorption materials were used for the removal of DAGs, MAGs, and the other polar components from frying oil, suggesting that the same process could be established for the removal of DAGs and MAGs from raw materials [132, 133]. Strijowski and his colleagues reported that approximately 25% of DAGs, MAGs, and the other polar components could be removed by amorphous magnesium silicate and calcined zeolite [134]. Craft and his colleagues reported that refined, bleached palm oil by either ethanol or glycerol during oil deodorization could remove the DAGs and FFAs, which resulted in a significant reduction of GEs and 3-MCPD esters [129].

7.4.3.2 Modification of Reaction Conditions

Temperature and reaction time represent the key factor of the reaction condition for the formation of GEs [123–125, 130].

Properly adjusting the temperature and reaction time of deodorization is critical in reducing the formation of GEs. Pudel and his colleagues reported that the GE content reduced to less than 5 ppm at a deodorization temperature lower than 240 °C [105]. However, when deodorization temperature increases to over 250 °C, the concentrations of GEs significantly increased in a time-dependent manner. Craft and

his colleagues confirmed that the formation of GEs significantly increased at a deodorization temperatures above 230 to 240 °C [135]. So, one possible method to inhibit the formation of GEs is to keep the deodorization temperature lower than 240 °C. Pudel and his colleagues carried out an experiment with a short-path distillation with a 60 °C condenser temperature, a 170 °C evaporator temperature, a 100 rpm stirrer speed, and a 20 Hz pump frequency [136]. The results of this study represented that with the mild deodorization condition, the content of GEs in refined edible oil decreased and the sensory quality (taste and odor) improved. Besides the temperature, reducing the reaction time is another important strategy. Pudel and his colleagues carried out an experiment with a two-stage deodorization; that is, the first step at a temperature of 250–270 °C in a short time and the second step at a temperature of 200 °C in a longer time [136]. Results represented that GE concentrations significantly reduced in this condition.

In addition, the oil fruit/seed pressing process also involves a roasting at a temperature of over 200 °C, which might induce GE formation and increase DAG and MAG contents [124]. Wong and his colleagues found that low levels of DAGs and MAGs and high temperature during the deep-frying reduced the formation of GEs in frying chicken breast meat [137]. Šmidrkal and his colleagues reported that the level of GEs formed is dependent on the pH value in oils [138]. An increase in pH value by addition of Na_2CO_3, $NaHCO_3$, or other alkaline substances could reduce the level of GEs formed in refined oils [107, 138–141]. However, the chemical mechanisms of pH in the reduction of GEs formed are still unclear and further experimental data are required.

Cheng and his colleagues found that the free radicals produced in the process of oil oxidation increase the GE formation [124]. And the 3-MCPD esters formed were reduced by using antioxidants in scavenging the free radicals [94, 142], based on the free radical formation mechanisms [99, 100]. Further studies are necessary to provide more evidence to support this hypothesis.

7.4.3.3 Elimination of Formed GEs

The elimination of formed GE methods includes two methods: physical adsorption and chemical degradation. The physical elimination methods are scavenging of the formed GEs by using activated carbon, magnesium silicate, zeolite, activated bleaching earth, or other adsorption materials [124]. Strijowski and his colleagues have reported the possibilities of a removal of GEs from palm oil by using different adsorption materials [134]. The results indicated that two of the adsorption materials (calcined zeolite and synthetic magnesium silicate) could reduce the formation of 3-MCPD esters and GEs up to 40%, and this physical elimination method did not show the adverse effect in palm oil, including oxidative stability and sensory properties.

Chemical degradation methods are adopted to transform GEs into other compounds, such as changing distillation process parameters or adding nontoxic reagents to refined oils to degrade GEs transforming GEs to glycerol, DAGs, MAGs,

or other nontoxic intermediates. Craft and his colleagues reported that adjusting the parameters of steam distillation steps could remove the high amounts of GEs (>100 mg/kg) in refined oils [143]. Özdikicierler and his colleagues investigated the effects of stripping steam rate, temperature, and pressure in the removal of the formation of GE in edible oil during the steam distillation process [144] and found that the level of GEs in olive oil and olive pomace oil decreases in the steam distillation temperature of 230 and 230 °C, the water flow rate of 1.2 mL/min and 1.0 mL/min, and the pressure of 4 mbar and 2 mbar, respectively. Besides, the new generated GEs are instable in acidic environment. Matthäus and his colleagues investigated the effects of acidic strip steam on GE formation by implementing formic acid in the oil deodorization step [104]. The results indicated that the content of GEs in refined edible oils decreases about 35% by the addition of higher concentration of formic acid in strip steam. Shimizu and his colleagues carried out an experiment to eliminate GEs by the addition of activated bleaching earth in DAG-rich oils in a model system [145]. And the results represented that the GE elimination effect of activated bleaching earth was not because of its physical adsorption effect but through the chemical conversion of GEs involving a ring-opening reaction.

In the past decades, a great deal of research had focused on the contamination of glycidol and GEs in food and food ingredients. The chemical mechanism research had shown the formation of GEs from various reagents during the food process. The analytical technique studies developed the effective and accurate quantification method for glycidol and GEs in food and food ingredients, allowing reliable assessments on glycidol and GE occurrence in the food processing.

7.5 Acrolein and Other Alkenals

Acrolein and other alkenals are a kind of electrophilic α,β-unsaturated aldehydes. The basic chemical structure of these compounds is an acrolein $CH_2 = CHCHO$ (Fig. 7.14). Acrolein is water soluble and could also be dissolved well in ethanol, acetone, and other organic solvents. Acrolein is colorless or pale yellow liquid and has a pungent odor like oil burning [146]. Acrolein and other alkenals are the important industrial raw materials, the major components of tobacco smoke, and could be released from the burned manufactured goods from the petroleum industry. Also, these compounds could be formed from overheated food lipids. Overcooked, fried, or charred foods are the major sources of acrolein and other alkenals. The TDI value (tolerable daily intake value) for acrolein is 7.5 μg/kg body weights [147].

Fig. 7.14 Chemical structure of acrolein and its fatty acid esters

Inhalation of acrolein can cause watery eyes, sore eyes, headaches, dizziness, coughing, and breathing problems. Moreover, acrolein could induce several diseases, including multiple sclerosis, spinal cord injury, Alzheimer's disease, diabetes mellitus, cardiovascular disease, and hepato-, nephro-, and neurotoxicity. On the cellular level, acrolein exposure could lead to DNA and protein adduction, oxidative and endoplasmic reticulum stress, mitochondria and membrane damage, and immune dysfunction [148]. Due to their wide existence of potential toxic effects, a systemic review about the analytical methods, formation mechanisms, and mitigation strategies of acrolein and other alkenals will be discussed in this section.

7.5.1 Analytical Methods

The analysis of acrolein in foods is extremely complex, so it requires great efforts. Early methods were based on thin and paper layer chromatography back to the 1960s or using spectroscopic techniques, such as UV-Vis and fluorescent spectroscopy, to detect the derivations of acrolein with hydroxylamine, N-methylhydrazine, morpholine, or sodium bisulfate. In the 1980s, HPLC-UV, GC-MS, and LC-MS have also been used for the determination of acrolein formed during heat processing of foods [147].

7.5.1.1 Chromatographic Methods in Detecting Acrolein

In 1987, acrolein from heated beef fat and cooking oils was analyzed by a gas chromatograph with a fused silica capillary column and a thermionic detector [149]. In 2009, Seaman and colleagues establish a method, which used gas chromatography-negative chemical ionization mass spectrometry and a labeled standard acrolein-d4 to quantify acrolein [150]. In 2011, two stable isotope dilution assays were developed for quantifying acrolein in fats and oils, which were a direct GC-MS headspace method and an indirect GC-MS method using derivation with pentafluorophenylhydrazine [151]. Also, in 2011, Osório and colleagues developed a GC-MS method to determine acrolein in French fries using SPME as the sampling technique after derivatization with 2,4-dinitrophenylhydrazine [152]. In 2012, an automatic method based on derivatization with 2,2,2-trifluoroethylhydrazine (2,2,2-TFEH) and consecutive headspace solid-phase microextraction and gas chromatography-mass spectrometry (HS-SPME/GC-MS) to detect acrolein in surface and drinking water are described [153]. In 2012, a fast qualitative and quantitative determination of acrolein in water was established by the portable headspace GC-MS analysis [154]. In 2013, Osório and colleagues used a method of solid-phase microextraction (SPME) coupled with gas chromatography and mass spectrometry to evaluate acrolein productions during frying the cassava and pork sausage in different vegetable oils [155]. In 2017, since using one-dimensional GC to analyze acrolein was difficult, a validated method using headspace solid-phase

microextraction (HS-SPME) combined with the comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC × GC/TOFMS) showed appropriate separation and identification abilities of the acrolein in wines [156]. In 2004, the liquid chromatographic separation equipped with pulsed electrochemical detection is described as a selective and sensitive analytical method for the determination of acrolein in heated vegetable oils [157]. In 2008, Uebori and colleagues determined acrolein in ambient air as its CNET derivative by LC/MS/MS [158].

7.5.1.2 Other Methods in Detecting Acrolein

In 1992, a membrane introduction mass spectrometry was used to directly quantify the acrolein in aqueous solution at low levels [159]. In 2016, Zheng and colleagues used nuclear magnetic resonance (NMR) spectroscopy to analyze acrolein degradation and established a quantitative headspace solid-phase microextraction/gas chromatography-mass spectrometry (HS-SPME/GC-MS) determination of acrolein in alcoholic beverages [160]. In 2017, a needle trap device (NTD) equipped with nanoporous silica aerogel (NPSA) as a sorbent was used as a new technique for analysis of acrolein. A 21-G needle was applied for extraction of gas in the sample headspace [161]. Also, in 2017, another NTD equipped with NPSA was developed as a technique for the rapid determination of acrolein and formaldehyde in air. Thus, this technique proved to be a reliable and effective method [162].

7.5.2 Formation Mechanisms

In 1916, the formation of acrolein was related with the oxidation of unsaturated fatty acids, particularly the linseed oil and linolenic acid [163]. In 1976, methional, the three-substituted propanal derived from methionine, was confirmed to easily decompose with the formation of acrolein with/without oxygen. Homoserine, homocysteine, and cystathionine could generate significant amounts of acrolein in aerobic interactions at neutral pH and 100 °C [164].

In 1992, acrolein was detected from cod liver oil upon ultraviolet irradiation (λ_{max} = 300 nm). It was derivative into nitrogen or sulfur-containing compounds and then analyzed by capillary gas chromatography equipped with a nitrogen-phosphorus detector or a flame photometric detector [165]. In 2010, a study reported that glycerol could be dehydrated to form acrolein, which has been mentioned in Alhanash’s research [166] (Figs. 7.15, 7.16, 7.17 and 7.18).

In 2013, several vegetable oils, including perilla, high-oleic sunflower, rice bran, soybean, and rapeseed oils, were heated at 180 °C for 480 min and then the concentration of acrolein was determined by gas-liquid chromatography.

In 2014, enzymatically synthesized linolenic and linoleic acid hydroperoxides were the key intermediates in acrolein formation.

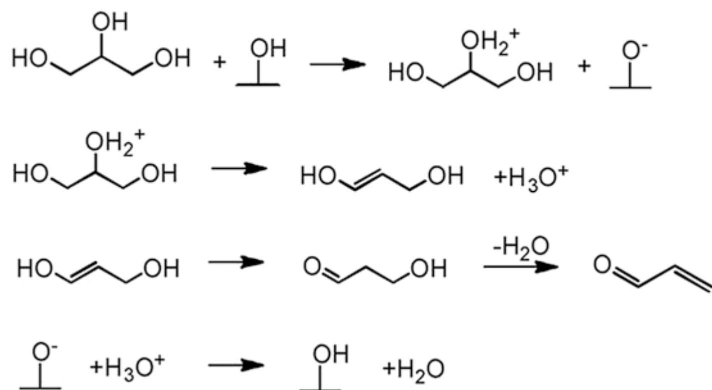

Fig. 7.15 Formation mechanism of acrolein esters from glycerol [166]

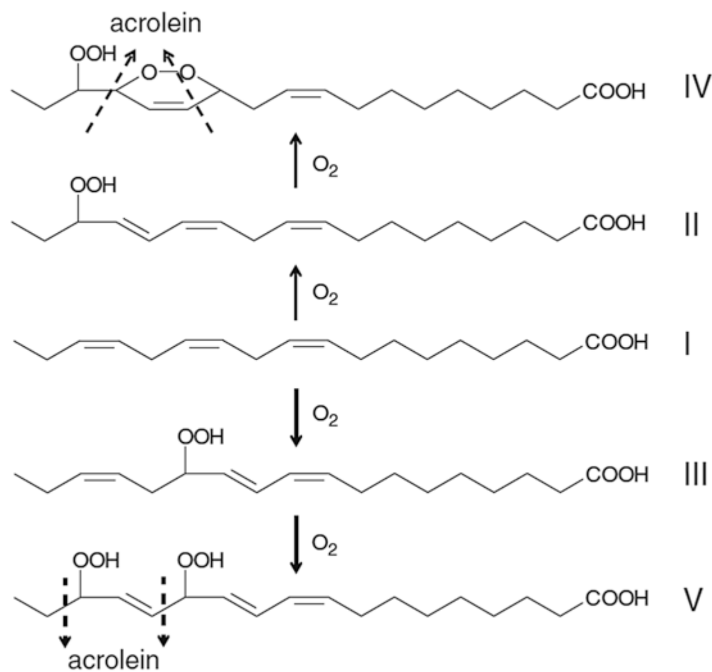

Fig. 7.16 Formation mechanism of acrolein esters from oil [167]

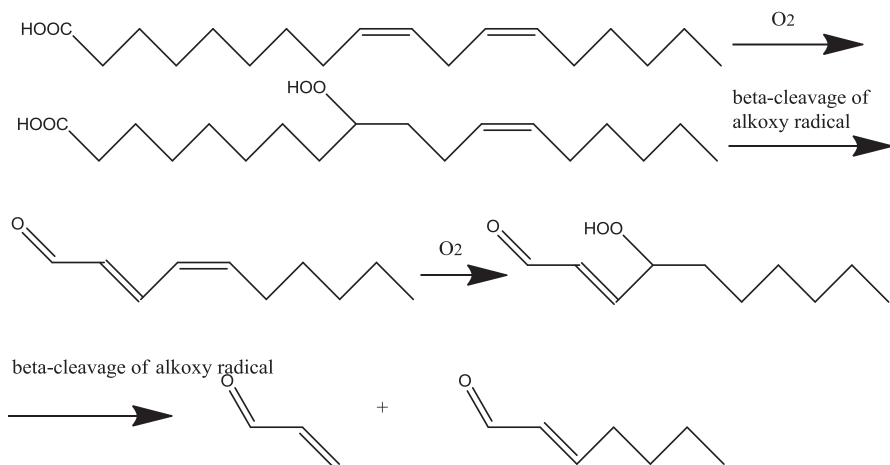

Fig. 7.17 Formation mechanism of acrolein esters from fatty acid [168]

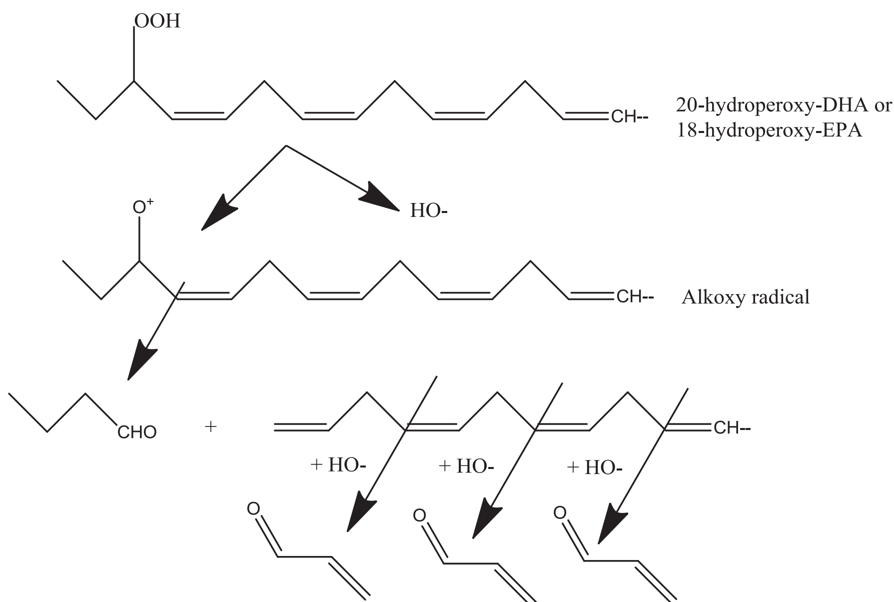

Fig. 7.18 Formation mechanism of acrolein esters from triacylglycerols [169]

In 2015, the high quantities of acrolein, in linseed, echium, fish, and soybean oil triacylglycerols oxidized at 50 or 60 °C, were detected by the static headspace gas chromatography method.

7.5.3 Mitigation Strategies

Acrolein, as a harmful and toxic substance in food, is a potential threat to human health. So, the removal of acrolein is extremely important. The mitigation strategies of acrolein could be separated into two major approaches, the direct removal and indirect removal.

7.5.3.1 Direct Removal Approaches

Direct removal approaches are the methods that reduce the acrolein with the acrolein scavenger directly. In 2004, Kaminskas and colleagues found the chemical mechanisms of acrolein trapping by hydralazine, and the study represented that together with its structural analogue dihydralazine, it also readily traps crotonaldehyde [170]. In 2006, Li and colleagues found that the nanoparticle dye is effective as an oxidant for the conversion of carbon monoxide to carbon dioxide and/or as a catalyst for the conversion of carbon monoxide to carbon dioxide and/or catalyst for conversion of aldehydes such as acetaldehyde and acrolein [171]. In 2011, Shi and colleagues also found that hydralazine serves as an excellent acrolein scavenger, since hydralazine can reduce acrolein concentrations and inhibit acrolein pathologies in vivo [115]. In 2012, PS-NH_2 was used to make active pharmaceutical ingredients (API) in the acrolein to get the most complete clearance. PS-NH_2 can remove 97.8% of acrolein without any substantial removal of the API during 20 min of reaction time. And in their API process solution system, scavenging of acrolein was seen to be quite fast and effective using both polymer- and silica-based scavengers. In addition, there are some nitrogen compounds that are capable of binding and inactivating acrolein [172]. In 2017, dimercaprol, which possesses thiol functional groups, was found to bind and trap acrolein and dimercaprol is not known to elicit a reduction in blood pressure. Therefore, in the event that hydralazine is not applicable in a subset of patients as a result of the risk of hypotension, dimercaprol could be a viable alternative treatment [173].

7.5.3.2 Indirect Removal Approaches

Indirect removal approaches refer to the inhibition of acrolein by not directly acting with acrolein, but by affecting its production or inhibiting the harmful physiological changes it produces. In 2011, Abraham and colleagues emphasized the strict control of the content of acrolein in food. For the exposure data, some improvements would be

necessary: development of valid analytical methods for the concentration of acrolein in foods, as well as the examination of foods in ready-to-eat form with high acrolein contents. The effective detection of acrolein in food could improve the control of acrolein by adjusting the processing materials [147]. In 2017, Gu and colleagues found that a squid solution polysaccharide (SIP) can effectively inhibit the Leydig cells in acrolein-mediated autophagy and apoptosis and thus play a role in scavenging [174].

This chapter discussed the analytical methods, formation mechanisms, and mitigation strategies of five groups of chemical hazards, including 5-HMF, trans-fatty acids, MCPDs and their esters, glycidol and its esters, acrolein, and other alkenals. The knowledge about these chemical hazards reported in food science in the past decades may improve our knowledge about these thermal processing-induced hazards and finally improve the food safety and quality in the thermal processing of foods.

References

1. Zhang XM et al (1993) Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethy1-2-furaldehyde in thermolyzed sucrose. Carcinogenesis 14(4):773–775
2. Surh YJ et al (1994) 5-Sulfooxymethylfurfural as a possible ultimate mutagenic and carcinogenic metabolite of the Maillard reaction product, 5-hydroxymethylfurfural. Carcinogenesis 15(10):2375–2377
3. Husøy T et al (2008) Dietary exposure to 5-hydroxymethylfurfural from Norwegian food and correlations with urine metabolites of short-term exposure. Food Chem Toxicol 46(12):3697–3702
4. SHINOHARA K (1986) Furans as the mutagens formed by amino-carbonyl reactions. Dev Food Sci 13:353–362
5. Janzowski C et al (2000) 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. Food Chem Toxicol 38(9):801–809
6. Program NT (2010) NTP toxicology and carcinogenesis studies of 5-(Hydroxymethyl)-2-furfural (CAS No. 67-47-0) in F344/N rats and B6C3F1 mice (gavage studies). Natl Toxicol Program Tech Rep Ser (554):7–13
7. Bauer-Marinovic M et al (2012) Toxicity studies with 5-hydroxymethylfurfural and its metabolite 5-sulphooxymethylfurfural in wild-type mice and transgenic mice expressing human sulphotransferases 1A1 and 1A2. Arch Toxicol 86(5):701–711
8. Pastoriza de la Cueva S et al (2017) Relationship between HMF intake and SMF formation in vivo: An animal and human study. Mol Nutr Food Res 61(3):1600773-n/a
9. Jöbstl D et al (2010) Analysis of 5-hydroxymethyl-2-furoic acid (HMFA) the main metabolite of alimentary 5-hydroxymethyl-2-furfural (HMF) with HPLC and GC in urine. Food Chem 123(3):814–818
10. Kocadağlı T et al (2012) In depth study of acrylamide formation in coffee during roasting: role of sucrose decomposition and lipid oxidation. Food Funct 3(9):970–975
11. Lo C-Y et al (2008) Reactive dicarbonyl compounds and 5-(hydroxymethyl)-2-furfural in carbonated beverages containing high fructose corn syrup. Food Chem 107(3):1099–1105
12. Oliviero T et al (2009) Influence of roasting on the antioxidant activity and HMF formation of a cocoa bean model systems. J Agric Food Chem 57(1):147–152
13. Rada-Mendoza M et al (2002) Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. Food Chem 79(4):513–516

14. Akpınar K et al (2011) Determination of HMF in roasted flour/oil mixtures and effect of solvent used in the extraction procedure. Food Chem 128(3):790–794
15. Durmaz G, Gökmen V (2010) Determination of 5-hydroxymethyl-2-furfural and 2-furfural in oils as indicators of heat pre-treatment. Food Chem 123(3):912–916
16. Arribas-Lorenzo G, Morales FJ (2010) Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population. Food Chem Toxicol 48(2):644–649
17. Chen Z, Yan X (2009) Simultaneous determination of melamine and 5-hydroxymethylfurfural in milk by capillary electrophoresis with diode array detection. J Agric Food Chem 57(19):8742–8747
18. del Campo G et al (2010) Quantitative determination of caffeine, formic acid, trigonelline and 5-(hydroxymethyl)furfural in soluble coffees by 1H NMR spectrometry. Talanta 81(1):367–371
19. Rajchl A et al (2013) Rapid determination of 5-hydroxymethylfurfural by DART ionization with time-of-flight mass spectrometry. Anal Bioanal Chem 405(14):4737–4745
20. de Andrade JK et al (2016) In house validation from direct determination of 5-hydroxymethyl-2-furfural (HMF) in Brazilian corn and cane syrups samples by HPLC–UV. Food Chem 190:481–486
21. Terra LR et al (2017) MCR-ALS applied to the quantification of the 5-Hydroxymethylfurfural using UV spectra: Study of catalytic process employing experimental design. Chemom Intel Lab Syst 167:132
22. Monien BH et al (2009) Conversion of the common food constituent 5-hydroxymethylfurfural into a mutagenic and carcinogenic sulfuric acid ester in the mouse in vivo. Chem Res Toxicol 22(6):1123
23. Kowalski S et al (2013) 5-Hydroxymethyl-2-furfural (HMF) – heat-induced formation, occurrence in food and biotransformation – a review. Pol J Food Nutr Sci 63(4):207–225
24. Antal MJ et al (1990) Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from d-fructose and sucrose. Carbohydr Res 199(1):91–109
25. Locas CP, Yaylayan VA (2008) Isotope labeling studies on the formation of 5-(hydroxymethyl)-2-furaldehyde (HMF) from sucrose by pyrolysis-GC/MS. J Agric Food Chem 56(15):6717
26. Capuano E, Fogliano V (2011) Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT Food Sci Technol 44(4):793–810
27. Brands CMJ, van Boekel MAJS (2003) Kinetic modelling of reactions in heated disaccharide–casein systems. Food Chem 83(1):13–26
28. Yaylayan VA, Huyghuesdespointes A (1994) Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. Crit Rev Food Sci Nutr 34(4):321
29. Wu S (2014) Glutathione suppresses the enzymatic and non-enzymatic browning in grape juice. Food Chem 160:8–10
30. Favreau-Farhadi N et al (2015) The inhibition of Maillard browning by different concentrations of Rosmarinic acid and epigallocatechin-3-gallate in model, bakery, and fruit systems. J Food Sci 80(10):C2140–C21C6
31. Oral RA et al (2014) Effects of certain polyphenols and extracts on furans and acrylamide formation in model system, and total furans during storage. Food Chem 142(1):423–429
32. Ameur LA et al (2006) Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: validation of an extraction method. Food Chem 98(4):790–796
33. Gökmen V et al (2007) Effects of dough formula and baking conditions on acrylamide and hydroxymethylfurfural formation in cookies. Food Chem 104(3):1136–1142
34. Taş NG, Gökmen V (2016) Effect of alkalization on the Maillard reaction products formed in cocoa during roasting. Food Res Int 89:930–936
35. Ameur LA et al (2007) Comparison of the effects of sucrose and hexose on furfural formation and browning in cookies baked at different temperatures. Food Chem 101(4):1407–1416

36. O'Brien J (1996) Stability of trehalose, sucrose and glucose to nonenzymatic browning in model systems. J Food Sci 61(4):679–682
37. Silvafernandes T et al (2017) Biodegradable alternative for removing toxic compounds from sugarcane bagasse hemicellulosic hydrolysates for valorization in biorefineries. Bioresour Technol 243:384
38. Doyle E (1997) Trans fatty acids. J Chem Educ 74(9):1030
39. Bansal G et al (2009) Analysis of trans fatty acids in deep frying oils by three different approaches. Food Chem 116(2):535–541
40. Khor GL, Esa NM (2008) trans Fatty acids intake: epidemiology and health implications. In: Trans fatty acids. Blackwell Publishing, Oxford, pp 25–45
41. Mensink RP, Katan MB (1990) Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. N Engl J Med 323(7):439–445
42. Zock PL, Katan MB (1992) Hydrogenation alternatives: effects of trans fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J Lipid Res 33(3):399–410
43. Nestel PJ et al (1992) Plasma cholesterol-lowering potential of edible-oil blends suitable for commercial use. Am J Clin Nutr 55(1):46–50
44. Judd JT et al (1994) Dietary trans fatty acids: effects on plasma lipids and lipoproteins of healthy men and women. Am J Clin Nutr 59(4):861–868
45. Willett WC et al (1993) Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 341(8845):581–585
46. Ascherio A et al (1996) Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. BMJ 313(7049):84–90
47. Seppänen-Laakso T et al (2002) Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition. Anal Chim Acta 465(1):39–62
48. Sherazi S et al (2009) Application of transmission FT-IR spectroscopy for the trans fat determination in the industrially processed edible oils. Food Chem 114(1):323–327
49. de Oliveira MA et al (2003) Method development for the analysis of trans-fatty acids in hydrogenated oils by capillary electrophoresis. Electrophoresis 24(10):1641–1647
50. Dobson G et al (1995) Silver ion chromatography of lipids and fatty acids. J Chromatogr B Biomed Sci Appl 671(1–2):197–222
51. Delmonte P, Rader JI (2007) Evaluation of gas chromatographic methods for the determination of trans fat. Anal Bioanal Chem 389(1):77–85
52. Ratnayake W et al (2002) Temperature-sensitive resolution of cis-and trans-fatty acid isomers of partially hydrogenated vegetable oils on SP-2560 and CP-Sil 88 capillary columns. J AOAC Int 85(5):1112–1118
53. Romero A et al (2000) Trans fatty acid production in deep fat frying of frozen foods with different oils and frying modalities. Nutr Res 20(4):599–608
54. Huang Z et al (2006) A simple method for the analysis of trans fatty acid with GC–MS and AT™-Silar-90 capillary column. Food Chem 98(4):593–598
55. de Oliveira MAL et al (2014) 20 years of fatty acid analysis by capillary electrophoresis. Molecules 19(9):14094–14113
56. Mossoba M et al (2007) Determination of total trans fats and oils by infrared spectroscopy for regulatory compliance. Anal Bioanal Chem 389(1):87–92
57. de Castro Barra PM et al (2013) An alternative method for rapid quantitative analysis of majority cis–trans fatty acids by CZE. Food Res Int 52(1):33–41
58. Porto BLS et al (2015) Fast screening method for the analysis of trans fatty acids in processed food by CZE-UV with direct detection. Food Control 55:230–235
59. Kim Y et al (2007) ATR-Fourier transform mid-infrared spectroscopy for determination of trans fatty acids in ground cereal products without oil extraction. J Agric Food Chem 55(11):4327–4333

60. Mahesar S et al (2010) Determination of total trans fat content in Pakistani cereal-based foods by SB-HATR FT-IR spectroscopy coupled with partial least square regression. Food Chem 123(4):1289–1293
61. Juanéda P (2002) Utilisation of reversed-phase high-performance liquid chromatography as an alternative to silver-ion chromatography for the separation of cis-and trans-C18: 1 fatty acid isomers. J Chromatogr A 954(1):285–289
62. Momchilova S et al (1998) Silver ion high-performance liquid chromatography of isomeric cis-and trans-octadecenoic acids: Effect of the ester moiety and mobile phase composition. J Chromatogr A 793(2):275–282
63. Destaillats F et al (2007) Comparison of available analytical methods to measure trans-octadecenoic acid isomeric profile and content by gas–liquid chromatography in milk fat. J Chromatogr A 1145(1):222–228
64. Adlof RO (1994) Separation of cis and trans unsaturated fatty acid methyl esters by silver ion high-performance liquid chromatography. J Chromatogr A 659(1):95–99
65. Adlof R, Lamm T (1998) Fractionation of cis-and trans-oleic, linoleic, and conjugated linoleic fatty acid methyl esters by silver ion high-performance liquid chromatography. J Chromatogr A 799(1):329–332
66. Kromer G (1975) Trends and patterns in soybean oil use for food and industrial products [World production, trade, consumption, statistics]. In: World Soybean Research conference, Champaign, Ill(USA), 3–8 Aug 1975; 1976: Interstate Printers and Publishers, Danville
67. Harfoot C, Hazlewood G (1997) Lipid metabolism in the rumen. The rumen microbial ecosystem, 2nd edn. Blackie Academic & Professional, London, pp 382–426
68. Kemp P et al (1975) The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including a new species. Microbiology 90(1):100–114
69. Kemp P, White R (1968) The biohydrogenation of linolenic and linoleic acids by bacteria isolated from an ovine rumen. Biochem J 106:55
70. Chen J et al (2001) Effects of conjugated linoleic acid on the degradation and oxidation stability of model lipids during heating and illumination. Food Chem 72(2):199–206
71. Balakos MW, Hernandez EE (1997) Catalyst characteristics and performance in edible oil hydrogenation. Catal Today 35(4):415–425
72. Allen RR, Kiess AA (1955) Isomerization during hydrogenation. I. Oleic acid. J Am Oil Chem Soc 32(7):400–405
73. Eckel RH et al (2007) Understanding the complexity of trans fatty acid reduction in the American diet. Circulation 115(16):2231–2246
74. Korver O, Katan MB (2006) The elimination of trans fats from spreads: how science helped to turn an industry around. Nutr Rev 64(6):275–279
75. Tarrago-Trani MT et al (2006) New and existing oils and fats used in products with reduced trans-fatty acid content. J Am Diet Assoc 106(6):867–880
76. Nishida C et al (2004) The Joint WHO/FAO Expert Consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. Public Health Nutr 7(1a):245–250
77. Leth T et al (2006) The effect of the regulation on trans fatty acid content in Danish food. Atheroscler Suppl 7(2):53–56
78. Astrup A (2006) The trans fatty acid story in Denmark. Atheroscler Suppl 7(2):43–46
79. Tan AS (2009) A case study of the New York City trans-fat story for international application. J Public Health Policy 30(1):3–16
80. Pérez-Ferrer C et al (2009) Learning from international policies on trans fatty acids to reduce cardiovascular disease in low-and middle-income countries, using Mexico as a case study. Health Policy Plan 25(1):39–49
81. Z Z et al (2006) Fatty acid esters of 3-chloropropane-1,2-diol in edible oils. Food Addit Contam 23(12):1290–1298
82. Baer I et al (2010) 3-MCPD in food other than soy sauce or hydrolysed vegetable protein (HVP). Anal Bioanal Chem 396(1):443–456

83. Z Z et al (2008) Occurrence of 3-MCPD fatty acid esters in human breast milk. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 25(6):669–676
84. Seefelder W et al (2008) Esters of 3-chloro-1, 2-propanediol (3-MCPD) in vegetable oils: significance in the formation of 3-MCPD. Food Addit Contam 25(4):391–400
85. Weißhaar R (2010) Determination of total 3-chloropropane-1,2-diol (3-MCPD) in edible oils by cleavage of MCPD esters with sodium methoxide. Eur J Lipid Sci Technol 110(2):183–186
86. Liu Q et al (2013) Simultaneous determination of total fatty acid esters of chloropropanols in edible oils by gas chromatography–mass spectrometry with solid-supported liquid–liquid extraction. J Chromatogr A 1314(11):208–215
87. Ermacora A, Hrncirik K (2013) A novel method for simultaneous monitoring of 2-MCPD, 3-MCPD and glycidyl esters in oils and fats. J Am Oil Chem Soc 90(1):1–8
88. Küsters M et al (2010) Rapid and simple micromethod for the simultaneous determination of 3-MCPD and 3-MCPD esters in different foodstuffs. J Agric Food Chem 58(11):6570–6577
89. Küsters M et al (2011) Simultaneous determination and differentiation of glycidyl esters and 3-monochloropropane-1,2-diol (MCPD) esters in different foodstuffs by GC-MS. J Agric Food Chem 59(11):6263–6270
90. Samaras VG et al (2016) Analytical method for the trace determination of esterified 3- and 2-monochloropropanediol and glycidyl fatty acid esters in various food matrices. J Chromatogr A 1466:136–147
91. Koyama K et al (2015) Collaborative study of an indirect enzymatic method for the simultaneous analysis of 3-MCPD, 2-MCPD, and glycidyl esters in edible oils. J Oleo Sci 64(10):557–568
92. Dubois M et al (2012) Comparison of indirect and direct quantification of esters of monochloropropanediol in vegetable oil. J Chromatogr A 1236(9):189–201
93. Andreoli R et al (2015) Quantification of 3-MCPD and its mercapturic metabolite in human urine: validation of an LC-MS-MS method and its application in the general population. Anal Bioanal Chem 407(16):4823–4827
94. Li H et al (2015) Direct determination of fatty acid esters of 3-chloro-1, 2-propanediol in edible vegetable oils by isotope dilution - ultra high performance liquid chromatography - triple quadrupole mass spectrometry. J Chromatogr A 1410:99–109
95. Macmahon S et al (2013) Analysis of processing contaminants in edible oils. Part 2. Liquid chromatography-tandem mass spectrometry method for the direct detection of 3-monochloropropanediol and 2-monochloropropanediol diesters. J Agric Food Chem 61(20):4748–4757
96. MacMahon S et al (2014) Liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the direct detection of 2-monochloropropanediol (2-MCPD) esters in edible oils. J Agric Food Chem 62(48):11647
97. Velisek J et al (2003) 3-chloropropane-1,2-diol in models simulating processed foods: precursors and agents causing its decomposition. Czech J Food Sci 21(5):153–161
98. Rahn AKK, Yaylayan VA (2015) What do we know about the molecular mechanism of 3-MCPD ester formation? Eur J Lipid Sci Technol 113(3):323–329
99. Zhang X et al (2013) Free radical mediated formation of 3-monochloropropanediol (3-MCPD) fatty acid diesters. J Agric Food Chem 61(10):2548–2555
100. Zhang Z et al (2015) Formation of 3-monochloro-1,2-propanediol (3-MCPD) di- and monoesters from tristearoylglycerol (TSG) and the potential catalytic effect of Fe^{2+} and Fe^{3+}. J Agric Food Chem 63(6):1839
101. Zhao Y et al (2016) Formation of 3-MCPD fatty acid esters from monostearoyl glycerol and the thermal stability of 3-MCPD monoesters. J Agric Food Chem 64(46):8918
102. Weißhaar R (2010) 3-MCPD-esters in edible fats and oils – a new and worldwide problem. Eur J Lipid Sci Technol 110(8):671–672
103. Hrncirik K, Van Duijn G (2015) An initial study on the formation of 3-MCPD esters during oil refining. Eur J Lipid Sci Technol 113(3):374–379

104. Matthäus B et al (2015) Strategies for the reduction of 3-MCPD esters and related compounds in vegetable oils. Eur J Lipid Sci Technol 113(3):380–386
105. Pudel F et al (2015) On the necessity of edible oil refining and possible sources of 3-MCPD and glycidyl esters. Eur J Lipid Sci Technol 113(3):368–373
106. Haines TD et al (2011) Direct determination of MCPD fatty acid esters and glycidyl fatty acid esters in vegetable oils by LC–TOFMS. J Am Oil Chem Soc 88(1):1–14
107. Freudenstein A et al (2013) Influence of precursors on the formation of 3-MCPD and glycidyl esters in a model oil under simulated deodorization conditions. Eur J Lipid Sci Technol 115(3):286–294
108. Sciences HDoP. NIOSH, manual of analytical methods: US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Division of Physical Sciences and Engineering; 1994
109. Miyazaki K et al (2012) Indirect method for simultaneous determinations of 3-chloro-1,2-propanediol fatty acid esters and glycidyl fatty acid esters. J Am Oil Chem Soc 89(8):1403–1407
110. AOCS J. Determination of bound monochloropropanediol-(MCPD-) and bound 2, 3-epoxy-1-propanol (glycidol-) by gas chromatography/mass spectrometry (GC/MS). Official Methods and Recommended Practices of the AOCS: AOCS Urbana (IL); 2013
111. AOCS J. JOCS Official Method Cd 29a-13: 2-and 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils and fats by acid transesterification. Official methods and recommended practices of the AOCS, 3rd printing. 2013; 2014
112. AOCS J. JOCS Official Method Cd 29c-13: Fatty-acidbound 3-chloropropane-1, 2, diol (3-MCPD) and 2, 3-epoxi-propane-1-ol (glycidol), Determination in oils and fats by gc/ms (differential measurement). Official methods and recommended practices of the AOCS, 3rd printing. 2013; 2014
113. Kuhlmann J (2015) Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound monochloropropanediol (MCPD) in refined oils. Eur J Lipid Sci Technol 113(3):335–344
114. Koyama K et al (2015) Optimization of an indirect enzymatic method for the simultaneous analysis of 3-MCPD, 2-MCPD, and glycidyl esters in edible oils. J Oleo Sci 64(10):1057–1064
115. Shi R et al (2011) Acrolein-mediated injury in nervous system trauma and diseases. Mol Nutr Food Res 55(9):1320–1331
116. Leigh JK, Macmahon S (2016) Extraction and liquid chromatography-tandem mass spectrometry detection of 3-monochloropropanediol esters and glycidyl esters in infant formula. J Agric Food Chem 64(49):9442
117. Hori K et al (2012) Simultaneous determination of 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils using liquid chromatography time-of-flight mass spectrometry. LWT Food Sci Technol 48(2):204–208
118. Dubois M et al (2011) Determination of seven glycidyl esters in edible oils by gel permeation chromatography extraction and liquid chromatography coupled to mass spectrometry detection. J Agric Food Chem 59(23):12291–12301
119. Masukawa Y et al (2010) A new analytical method for the quantification of glycidol fatty acid esters in edible oils. J Oleo Sci 59(2):81–88
120. Steenbergen H et al (2013) Direct analysis of intact glycidyl fatty acid esters in edible oils using gas chromatography–mass spectrometry. J Chromatogr A 1313:202–211
121. Song Z et al (2015) A novel 1H NMR spectroscopic method for determination of glycidyl fatty acid esters coexisting with acylglycerols. Eur J Lipid Sci Technol 117(7):918–925
122. Destaillats F et al (2012) Formation mechanisms of monochloropropanediol (MCPD) fatty acid diesters in refined palm (Elaeis guineensis) oil and related fractions. Food Addit Contam 29(1):29–37
123. Weißhaar R, Perz R (2010) Fatty acid esters of glycidol in refined fats and oils. Eur J Lipid Sci Technol 112(2):158–165

124. Cheng WW et al (2016) Formation of glycidyl fatty acid esters both in real edible oil during laboratory-scale refining and in chemical model during high temperature exposure. J Agric Food Chem 64(29):5919
125. Destaillats F et al (2012) Glycidyl esters in refined palm (Elaeis guineensis) oil and related fractions. Part I: Formation mechanism. Food Chem 131(4):1391–1398
126. Kopas PM, Kopas G (2009) Cosmeceutical formulation containing palm oils. Google patents
127. Sambanthamurthi R et al (1995) Factors affecting lipase activity in the oil palm (*Elaeis guineensis*) mesocarp. In: Plant Lipid Metabolism. Springer, Dordrecht, pp 555–557
128. Cadena T et al (2013) Lipase activity, mesocarp oil content, and iodine value in oil palm fruits of Elaeis guineensis, Elaeis oleifera, and the interspecific hybrid O × G (E. oleifera × E. guineensis). J Sci Food Agric 93(3):674–680
129. Craft BD et al (2012) Factors impacting the formation of monochloropropanediol (MCPD) fatty acid diesters during palm (*Elaeis guineensis*) oil production. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 29(3):354–361
130. Stadler RH (2015) Monochloropropane-1, 2-diol esters (MCPDEs) and glycidyl esters (GEs): an update. Curr Opin Food Sci 6:12–18
131. Aniołowska MA, Kita AM (2016) The effect of raw materials on thermo-oxidative stability and glycidyl esters content of palm oil during frying. J Sci Food Agric 96(6):2257–2264
132. Lin S et al (2001) Recovery of used frying oils with adsorbent combinations: refrying and frequent oil replenishment. Food Res Int 34(2):159–166
133. Yates RA, Caldwell JD (1993) Regeneration of oils used for deep frying: a comparison of active filter aids. J Am Oil Chem Soc 70(5):507–511
134. Strijowski U et al (2011) Removal of 3-MCPD esters and related substances after refining by adsorbent material. Eur J Lipid Sci Technol 113(3):387–392
135. Craft BD, Nagy K (2012) Mitigation of MCPD-ester and glycidyl-ester levels during the production of refined palm oil. Lipid Technol 24(7):155–157
136. Pudel F et al (2016) 3-MCPD- and glycidyl esters can be mitigated in vegetable oils by use of short path distillation. Eur J Lipid Sci Technol 118(3):396–405
137. Wong YH et al (2017) Effects of temperature and NaCl on the formation of 3-MCPD esters and glycidyl esters in refined, bleached and deodorized palm olein during deep-fat frying of potato chips. Food Chem 219:126–130
138. Šmidrkal J et al (2011) Formation of acylglycerol chloro derivatives in vegetable oils and mitigation strategy. Czech J Food Sci 29(4):448–456
139. Sim CW et al (2004) The optimization of conditions for the production of acid-hydrolysed winged bean and soybean proteins with reduction of 3-monochloropropane-1,2-diol (3-MCPD). Int J Food Sci Technol 39(9):947–958
140. MR R et al (2015) Other factors to consider in the formation of chloropropandiol fatty esters in oil processes. Food Addit Contam 32(6):817–824
141. Šmidrkal J et al (2016) Mechanism of formation of 3-chloropropan-1,2-diol (3-MCPD) esters under conditions of the vegetable oil refining. Food Chem 211:124–129
142. Zhang H et al (2016) Mitigation of 3-monochloro-1,2-propanediol ester formation by radical scavengers. J Agric Food Chem 64(29):5887–5892
143. Craft BD et al (2012) Glycidyl esters in refined palm (Elaeis guineensis) oil and related fractions. Part II: Practical recommendations for effective mitigation. Food Chem 132(1):73–79
144. Özdikicierler O et al (2016) Effects of process parameters on 3-MCPD and glycidyl ester formation during steam distillation of olive oil and olive pomace oil. Eur Food Res Technol 242(5):805–813
145. Shimizu M et al (2012) Elimination of glycidyl palmitate in diolein by treatment with activated bleaching earth. J Oleo Sci 61(1):23–28
146. Moretto N et al (2012) Acrolein effects in pulmonary cells: relevance to chronic obstructive pulmonary disease. Ann N Y Acad Sci 1259(1):39–46
147. Abraham K et al (2011) Toxicology and risk assessment of acrolein in food. Mol Nutr Food Res 55(9):1277–1290

148. Moghe A et al (2015) Molecular mechanisms of acrolein toxicity: relevance to human disease. Toxicol Sci 143(2):242–255
149. Umano K, Shibamoto T (1987) Analysis of headspace volatiles from overheated beef fat. J Agric Food Chem 35(1):14–18
150. Seaman VY (2009) Indoor acrolein emission and decay rates resulting from domestic cooking events. Atmos Environ 43(39):6199–6204
151. Ewert A et al (2011) Development of two stable isotope dilution assays for the quantitation of acrolein in heat-processed fats. J Agric Food Chem 59(8):3582
152. Osório VM, De LCZ (2011) Determination of acrolein in french fries by solid-phase microextraction gas chromatography and mass spectrometry. J Chromatogr A 1218(21):3332–3336
153. Lim HH, Shin HS (2012) Simple determination of acrolein in surface and drinking water by headspace SPME GC–MS. Chromatographia 75(15–16):943–948
154. Deng MJ et al (2012) Fast determination of acrolein and acrylonitrile in water by the portable GC-MS and selected ion monitoring. Environ Monit China 28(5):71–73
155. Osório VM, Cardeal ZL (2013) Using SPME-GC/MS to evaluate acrolein production in cassava and pork sausage fried in different vegetable oils. J Am Oil Chem Soc 90(12):1795–1800
156. Lago LO et al (2017) Influence of ripeness and maceration of the grapes on levels of furan and carbonyl compounds in wine – simultaneous quantitative determination and assessment of the exposure risk to these compounds. Food Chem 230:594–603
157. Casella IG, Contursi M (2004) Quantitative analysis of acrolein in heated vegetable oils by liquid chromatography with pulsed electrochemical detection. J Agric Food Chem 52(19):5816–5821
158. Uebori M et al (2008) Determination of acrolein in ambient air as it's cnet derivative by lc/ms/ms. J Environ Chem 18(1):73–80
159. Choudhury TK et al (1992) Analysis of acrolein and acrylonitrile in aqueous solution by membrane introduction mass spectrometry. Talanta 39(9):1113
160. ZHENG J et al (2016) Determination of acetaldehyde, acrolein, acrylonitrile and pyridine in water with headspace gas chromatography. J Guizhou Norm Univ (Nat Sci) 3:017
161. Barkhordari A et al (2017) Analysis of formaldehyde and acrolein in the aqueous samples using a novel needle trap device containing nanoporous silica aerogel sorbent. Environ Monit Assess 189(4):171
162. Azari MR et al (2017) A novel needle trap device with nanoporous silica aerogel packed for sampling and analysis of volatile aldehyde compounds in air. Microchem J 134:270–276
163. Salway AH (1916) XII.—Studies on the oxidation of unsaturated fatty oils and unsaturated fatty acids. Part I. The formation of acrolein by the oxidation of linseed oil and linolenic acid. J Chem Soc Trans 109:138–145
164. Alarcon RA (1976) Formation of acrolein from various amino-acids and polyamines under degradation at 100 degrees C. Environ Res 12(3):317–326
165. Niyati-Shirkhodaee F, Shibamoto T (1992) Formation of toxic aldehydes in cod liver oil after ultraviolet irradiation. J Am Oil Chem Soc 69(12):1254–1256
166. Alhanash A et al (2010) Gas-phase dehydration of glycerol to acrolein catalysed by caesium heteropoly salt. Appl Catal A Gen 378(1):11–18
167. Endo Y et al (2013) Linolenic acid as the main source of acrolein formed during heating of vegetable oils. J Am Oil Chem Soc 90(7):959–964
168. Ewert A et al (2014) Isotope-labeling studies on the formation pathway of acrolein during heat processing of oils. J Agric Food Chem 62(33):8524–8529
169. Shibata A et al (2015) Formation of acrolein in the autoxidation of triacylglycerols with different fatty acid compositions. J Am Oil Chem Soc 92(11–12):1661–1670
170. Kaminskas LM et al (2004) Reactivity of hydrazinophthalazine drugs with the lipid peroxidation products acrolein and crotonaldehyde. Org Biomol Chem 2(18):2578–2584
171. Ping LI, et al (2002) Oxidant/catalyst nanoparticles to reduce tobacco smoke constituents such as carbon monoxide

172. Kecili R et al (2012) Removal of acrolein from active pharmaceutical ingredients using aldehyde scavengers. Org Process Res Dev 16(6):1225–1229
173. Tian R, Shi R (2017) Dimercaprol is an acrolein scavenger that mitigates acrolein-mediated PC-12 cells toxicity and reduces acrolein in rat following spinal cord injury. J Neurochem 141(5):708–720
174. Gu YP et al (2017) Inhibition of acrolein-induced autophagy and apoptosis by a glycosaminoglycan from *Sepia esculenta* ink in mouse Leydig cells. Carbohydr Polym 163:270–279